

Abstracts

PL01. Garrod's inborn errors of metabolism: Lessons from a defective heme pathway

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Heme, iron-containing tetrapyrrole, is indispensable for life. It is utilized by a whole host of proteins involved in numerous cellular processes such as oxygen transport (hemoglobin), respiration (cytochrome oxidase), vascular homeostasis (nitric oxide synthases), detoxification (cytochromes P450), and cell death (cytochrome c). Heme is produced in the mitochondrion by a complex cellular machinery comprising eight enzymes that are evolutionarily conserved from bacteria to humans. Mutations in genes encoding the heme biosynthetic pathway enzymes lead to diseases broadly classified as porphyrias. In the past two decades these genes have been cloned and the disease-causing mutations have been identified. We have established a web site (www.porphyria_europe.com) that details the efforts to diagnose and treat porphyrias. In this presentation I will focus on the antepenultimate step of this pathway in which deficiency of coproporphyrinogen oxidase (CPO) activity results in hereditary coproporphyrina(HCP)-an autosomal dominant disorder characterized by acute attacks (severe abdominal pain, neuropsychiatric symptoms, and / or skin lesions). First, we cloned the cDNA and characterized the gene (3q11.2) encoding CPO. Next, we purified, crystallized, and determined the novel structure of CPO to gain insights into the structural basis of HCP. Thus, mutations primarily destabilize molecular interactions that are critical for activity. Since prokaryotes employ two different gene products to mediate CPO activity, we hypothesized that an ortholog of the second bacterial gene may be found in humans. A bioinformatics approach validated this prediction and led to the discovery of a gene called RSAD1 (radical S-adenosyl methionine domain 1). Interestingly, RSAD1's mitochondrial function is not redundant with CPO. We have since discovered that it is involved in cardiovascular development. Therefore, a detailed study of heme biosynthetic pathway enzymes have not only resulting in molecular insights into porphyrias, but have provided leads regarding mitochondrial homeostasis mechanisms.

PL02. Garrod's inborn errors of metabolism: Lessons from homocystinuria

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Four decades ago homocystinuria due to cystathione beta-synthase (CBS) has been described as a typical inborn error of metabolism partially resembling the Marfan syndrome. As extremely high concentrations of plasma homocysteine result in vascular pathology, mild hyperhomocysteinemia has been subsequently considered a risk factor for arteriosclerosis and other complex diseases. In the past ten years our Prague team had an opportunity to explore selected facets of CBS deficiency, some of which are discussed below.

The CBS gene-located at chromosome 21- spans 28 kbp and contains numerous non-pathogenic variants with population specific frequencies. Analyses of more than 550 patient-derived CBS alleles worldwide revealed over 130 different pathogenic mutations, which are deposited at <http://www.uchsc.edu/cbs>. As pathogenic mechanisms in CBS deficiency are only partially understood a substantial part of our work was aimed at exploring molecular consequences of selected mutants: i/we observed that some - but not all- RNA molecules carrying premature termination codons are unstable owing to the rapid degradation by nonsense-mediated decay; ii/our current work is focused on studying misfolding of mutants as a common mechanism in homocystinuria. Homocystinuria is traditionally perceived as a rare disease with worldwide incidence of 1:355,000. However, recent studies based on detecting heterozygotes for selected mutations in anonymous newborn samples suggest that CBS deficiency may be considerably more common throughout Europe with expected birth prevalence of homozygotes ranging between 1:6,400 in Norway and 1:15,000 in the Czech Republic.

Intense research of CBS deficiency- a supposedly rare inborn error

of sulfur metabolism- enriched the knowledge of human biology in at least two areas: i/it identified homocysteine as a possible risk factor for common complex diseases such as arteriosclerosis; ii/ epidemiological studies suggests that the common practice may in fact identify only a fraction of individuals affected by this diseases, which calls for re-evaluation of newborn screening programs.

PL03. TEL/AML1 fusion gene in childhood acute lymphoblastic leukaemia

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Acute lymphoblastic leukaemia (ALL) is the most common malignancy in childhood. The cryptic translocation t(12;21), discovered only in 1994 and resulting in TEL/AML1 chimaeric gene, is the single most frequent non-random chromosomal aberration in childhood ALL (~25%). It was shown to be specifically related to the leukaemias with the onset in the pre-school age. Twin and backtracking studies showed that TEL/AML1-positive leukaemias originate in utero and the TEL/AML1 fusion was later identified as a first leukaemogenic event. The analysis of the non-translocated TEL allele deletions proposed this aberration as an ultimate leukaemia-triggering hit. Both partner genes of the fusion are important for the development and maintenance of normal haematopoiesis. The TEL part of the fusion protein contains domains interacting with mSin3A, N-CoR and HDAC-3 corepressors. Consequently, TEL/AML1 is supposed to negatively regulate the expression of genes that, under normal conditions, are transactivated by AML1, thus affecting the differentiation during haematopoiesis by chromatin remodelling via association with histone deacetylases (HDAC). Indeed, TEL/AML1-positive cells show dose-dependent arrest in proliferation and also the maturation drift after treatment with HDAC inhibitors, thus supporting this hypothesis.

The prognosis of children with TEL/AML1-positive ALL tends to be better-than-average, however, relapses occur also in this subgroup in approximately 15% of children. Since the response to the initial treatment is the main predictor of outcome in childhood ALL, quantitative detection of TEL/AML1 transcript in early phases of treatment can serve as suitable tool for the risk stratification of patients. Detectable minimal residual disease predicts relapse in children with TEL/AML1-positive ALL both on front-line treatment and after stem cell transplantation. These findings have important clinical implications for the determination of prognosis and potentially also for the treatment of 25% of children with ALL. They also represent the progress in the field of paediatric acute leukaemias during the past decade.

PL04. New Mouse Model of Human Aneuploidy syndrome

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Aneuploidy syndromes, such as Down syndrome, represent complex developmental disorders with the known cause, but with largely unknown mechanisms that link the triplicated chromosome with abnormal developmental/physiological traits. The hypothesis of dosage-sensitive genes explains the abnormal phenotype as a specific consequence of elevated expression of a subset of triplicated genes, while the amplified developmental instability hypothesis posits a rather non-specific effect of a large number of overexpressed genes. One way to distinguish between these two hypotheses is to compare, at the phenotype and gene expression levels, unrelated partial trisomies that differ in size as well as in the number of triplicated genes. The existing mouse models are not sufficient in this respect because they triplicate overlapping subsets of orthologs to human chromosome 21. In this study we investigated an independent mouse model of segmental trisomy, Ts43H, which triplicates 30 Mb of proximal chromosome 17, encompassing >300 known genes of ENSEMBL 29 database. This trisomy shows a limited overlap with Ts65Dn segmental trisomy (40-70 genes) and no overlap with other mouse models. The Ts43H mice exhibited spatial learning deficits in Morris Water Maze similar to those observed in Ts65Dn. qRT PCR of the brain expression of 20 genes inside the trisomic interval and of 12 genes lying outside on Chr17

revealed 1.2-fold average increase of mRNA levels of triplicated genes and 0.9-fold average downregulation of genes beyond the border of trisomy. We propose that systemic comparison of gene expression levels with global phenotype (phenome) data between unrelated segmental trisomies, such as Ts65Dn and Ts43H, will help to disclose the affected functional networks leading from the triplicated sequences to the complex developmental anomalies.

Vacík, T., Ort, M., Gregorová, S., Strnad, P., Blatný, R. Conte, N., Bradley, A., Bureš, J., Forejt. *J.: Proc. Natl. Acad. Sci. U.S.A.* 102:4500-4505 2005.

PL05. Pharmacogenetics of drug metabolising enzymes. Implications for a safer and more efficient drug therapy

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Drug treatment is in many cases ineffective. Non responders and patients suffering from adverse drug reactions is estimated to cost the US society 100 billion USD and over 100,000 deaths per year. Many drug transporters are polymorphic. In addition, the majority of phase I and phase II dependent drug metabolism is carried out by polymorphic enzymes which can cause abolished, quantitatively or qualitatively altered or enhanced drug metabolism. Stable duplication, multiduplication or amplification of active genes, most likely in response to dietary components that have resulted in a selection of alleles with multiple non-inducible genes, has been described. Several examples exist where subjects carrying certain alleles suffer from a lack of drug efficacy due to ultrarapid metabolism caused by multiple genes or by induction of gene expression, or, alternatively, adverse effects from the drug treatment due to the presence of defective alleles. The evolutionary aspect of the genetic polymorphism in these genes includes genetic drift but also selection because of environmental stress. The information about the role of polymorphic drug transporters and drug receptors for efficiency of drug therapy is scarcer, although promising examples are seen in drug treatment of e.g. asthma. In addition, certain polymorphic genes can be used as markers for optimisation of the drug therapy. It is likely that predictive genotyping in the future will be of benefit in 20-30 % of drug treatment and thereby allows for prevention of causalities as a cause of ADRs and thus improves the health for a significant fraction of the patients. Recently several important clinical examples have been given. In the lecture an overview of the current status in the field will be given.

PL06. The future of pharmacogenetics

A. Roses:

Genetics Research, GlaxoSmithKline, Research Triangle Park, NC, United States.

No abstract received.

PL07. Advances in human genetics: what benefits for the patients?

A. Munnich:

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The recent advances of human genetics have raised considerable hopes for a better future of patients with genetic diseases. Yet, apart from early diagnosis and prevention, have disease gene identification improved their condition and changed their life? In fact, while gene therapy has riveted most of attention/investments with hitherto limited impact on their condition, it appears that understanding the disease mechanisms has occasionally fostered spectacular improvement in their lifespan and quality of life. This includes 1) the dietary management of recently identified metabolic diseases (CDG Ib), 2) their occasional responsiveness to vitamins, cofactors (quinones, carnitine) and lacking compounds (creatine-responsive mental retardation), 3) organ transplantation (kidney, liver, heart, bone marrow) and neuro-electrostimulation (torsion dystonia, DYT1), 4) protein engineering (hemophilia, diabetes, growth hormone deficiency, congenital adrenal hyperplasia), 5) enzyme replacement therapy (Gaucher, Fabry, Pompe, Hurler disease), and, 6) conventional pharmacology. Indeed, elucidating the molecular bases of diseases has led to successful re-expression of fetal hemoglobin by hydroxyurea for thalassemias and sickle cell anemia and improvement of periodic familial fever

by colchicine, to the continuation of translation by gentamycin in cystic fibrosis and hopefully to correction of abnormal splicing by antisense RNA in Duchenne muscular dystrophy (at least *in vitro*). Similarly, it is now possible 1) to clear a toxic compound (benzoate in urea cycle diseases, cysteamine in cystinosis), 2) to lock an impaired pathway (NTBC in tyrosinemia type 1), 3) to activate a pathway (fibrates in carnitine palmitoyl transferase 2 deficiency), 4) to inhibit a function (bisphosphonates in osteogenesis imperfecta), 5) to replace a function (melatonin in Smith Magenis syndrome), or to protect a targeted function (idebenone in free-radical induced oxphos injury in Friedreich ataxia). Based on this variety of procedures, it is clear that understanding the exact disease mechanism can help devising original therapeutic approaches to fight genetic diseases. One should not ignore any of these approaches and one should not put "all his eggs in one basket". Finally, while genotyping is not presently required for diagnosing a genetic disease, identifying the disease causing mutation (splicing, nonsense..) might soon become mandatory to devise "à la carte" therapeutic strategies.

PL08. Asthma

J. Kere:

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No abstract received.

PL09. Genes and mechanisms in type 1 diabetes

J. Todd:

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At least 90% of cases of type 1 diabetes who are absolutely insulin-dependent since diagnosis suffer from an autoimmune destruction of the insulin-producing beta cells of the pancreas. There are four confirmed susceptibility genes in type 1 diabetes, the HLA class II complex, insulin (*INS*), *CTLA4* and *PTPN22*, with evidence for a fifth, mapping to the CD25 region (Vella, A et al *Am J Hum Genet*, 76, Epub, 2005). The known functions of these four genes, and their unequivocal association with the disease, indicates that a major pathway in beta-cell destruction is the breakdown in immune tolerance to preproinsulin. Further gene discovery will identify what other pathways are involved (Wang, W et al *Nat Rev Genet*, 6, 109-118, 2005). For example, we have evidence that the HLA class I gene, *HLA-B*, is associated independently of the class II genes, which would substantiate the assumed direct involvement of CD8 cytotoxic T cells in disease and could perhaps implicate natural killer cell activity. Knowledge of gene association has also begun to help in the first steps towards sub-classification of type 1 diabetes: we have found that 11% of paediatric cases of type 1 diabetes have autoantibodies to the autoimmune thyroid disease (AITD)-associated antigen, thyroid peroxidase, and that the T cell immunoregulatory *CTLA-4* gene acts almost exclusively in this smaller subgroup of patients. These results indicate that AITD-complicated type 1 diabetes is different in an aetiological way to the isolated form of the disease, in which the effects of the soluble form of *CTLA-4* (Ueda, H et al *Nature* 423, 506-511, 2003) are greatly reduced, perhaps by the action in these cases of other, as yet undiscovered genes, that bypass or mask the negative T cell signalling component of the AITD-complicated sub-class of the disease.

PL10. Genetics of Crohn disease, an archetypal inflammatory barrier disease

S. Schreiber:

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Chronic inflammatory disorders such as Crohn disease, atopic eczema, asthma, and psoriasis are triggered by hitherto unknown environmental factors, acting on the background of some polygenic susceptibility. They share the common theme of inflammation of barrier organs. This includes similarities in clinical manifestations, a chronic relapsing character of disease and a striking overlap in disease pathophysiology. Recent technological advances have allowed to unravel the genetic etiology of these and other complex diseases. Using Crohn disease as an example, it will be shown how the discovery of susceptibility

genes furthers our understanding of the disease mechanisms involved and, ultimately, will give rise to new therapeutic developments. The long-term goal of such endeavors is to develop targeted prophylactic strategies that most likely will concern the molecular interaction on the mucosal surface between the genome and the microbial metagenome of a patient.

PL11. Identifying the genes encoding longevity

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Each species has its own characteristic life span but within a species individual life spans vary widely. This variation is in part the result of an individual ability to avoid or cope with internal and external damage. For example, single point mutations in the insulin-signaling pathway of *C. elegans* can lower the rate of aging and lengthen life span up to nearly five times as long as the wild type worms. These experimental data suggest that a major part of the age-related changes are under coordinated genetic control. Twin studies have shown that approximately 25% in the variation of human lifespan is explained by genetic factors.

The search into the genetic loci that explain the inter-individual differences in human longevity has peculiar characteristics. By virtue of the extremeness of the phenotype, linkage studies in extended families are cumbersome as members are often too young to determine their phenotypic status, whereas others whose status is beyond doubt have already deceased. An ordinary association study also generates problems as the long-lived cases originate from a birth cohort that has never been defined and of which the controls have already deceased. When a younger control group is used for comparison a particular form of bias is introduced as these have been selected for fertility over two generations. Evolutionary theory predicts that a genetic predisposition for fertility has a cost at survival. Haplotypes that associate with fertility and a shorter life are thus, by design, increased in the control group. Two alternative approaches are presented: (1) investigating linkage in an affected sib-pair design, and (2) assessment of association in a classic prospective follow-up design. Recent studies have demonstrated a clustering of extreme longevity within families and localized a locus on chromosome 4. These data will be discussed in the light of the outcomes of the Leiden Longevity Study that also uses linkage in an affected sib pair design. Finally, data will be presented on genetic variation in the evolutionary conserved insulin-signaling pathway from the prospective Leiden 85-plus study.

PL12. IGF signalling and aging

M. Holzenberger:

Inserm U515, Hôpital Saint-Antoine, Paris, France.

No abstract received.

PL13. Genetics of early and accelerated ageing syndromes

N. Levy:

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Segmental aging disorders have been characterized at the clinical level for many years. Most of them were shown to be due to alterations of DNA repair/replication/recombination/transcription mechanisms. Among these latter, autosomal recessive Werner syndrome, caused by mutations in RecQL2 encoding a DNA helicase, has been widely explored and is the better characterized at the clinical molecular and cellular level. More recently, a distinct set of premature and accelerated segmental aging syndromes has been uncovered, due to Lamins A/C defects. Lamins A/C are ubiquitous nuclear proteins belonging to the intermediate filament family; mutations in *LMNA*, encoding them, are responsible of different allelic disorders, known as "laminopathies", varying in severity from mild to lifespan-reducing/neonatal lethal syndromes. Lamin-related segmental progeroid syndromes include, increasing in severity: lipodystrophy-atypical Werner syndromes (LIRLLC, WS), Mandibuloacral Dysplasia (MAD), Hutchinson-Gilford Progeria syndrome (HGPS) and Restrictive Dermopathy (RD). These syndromes are characterized by alterations of Lamins A/C expression levels, function and distribution which can either be primary, due to *LMNA* pathogenic sequence variations, or

in MAD and RD, secondary, due to defects in *ZMPSTE24*, encoding a metalloproteinase involved in processing of Lamin A precursors. While *LMNA* mutations responsible of most laminopathies, as well as of the less severe segmental progeroid syndromes LIRLLC, atypical WS, MAD, affect both Lamins A and C, *LMNA* mutations responsible of the more severe HGPS and RD, specifically affect Lamin A. HGPS and RD have recently been shown to be due to intranuclear accumulation of unprocessed, truncated or wild type, prelamin A, respectively due to intrinsic lack of key post-translational processing sites or lack of *ZMPSTE24*. These accumulated precursors seem to have dominant negative effects on residual wild type proteins' function. Furthermore, it has been recently showed *in vitro* and *in vivo*, that a reduction of the amounts of prelamin A produced by the cells can spectacularly reverse the cellular pathological phenotype. These observations constitute indeed an exciting hope towards targeted molecular therapeutic strategies in patients.

S01. Systems biology in cardiovascular disease

F. Cambien:

INSERM U525, University Paris VI 'Pierre et Marie Curie', Paris, France.

No abstract received.

S02. Bioinformatics of signalling pathways

R. Eils:

Division of Theoretical Bioinformatics, German Cancer Research Center (DKFZ), Heidelberg, Germany.

No abstract received.

S03. Integral Membrane Proteins and Visual Defects

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The visual system in humans is prone to arguably the widest range of human genetic defects leading to different levels of visual impairment. Examples include various forms of colour blindness and retinitis pigmentosa. Several proteins are subject to mutational change. This presentation will focus on two most heavily implicated in the machinery of light capture-rhodopsin and peripherin, both integral membrane proteins in the discs of the rod cell.

There are four visual opsins in the human genome, each of which combines with 11-cis retinal to generate the pigments responsible for colour vision and vision under conditions of dim light. Colour blindness results from the absence of colour pigment genes whilst mutations in rhodopsin give rise to a number of different conditions some of which result in blindness. The 3-D structure of rhodopsin is known: thus this presentation will provide structural explanations as to how mutational changes could impair the function of the protein and consequently give rise to the clinical effect.

The role of peripherin is less clear but mutations in this protein give rise to the degeneration of the retina. Topographical mapping using glycosylation has established that the protein probably consists of 4 transmembrane regions and a large loop which is located in the intradiscal (intraluminal) space of the retina discs. Most of the clinically meaningful mutations in peripherin are located in this loop region. A probable role for the protein in causing the invagination and flattening of the plasma membrane to give the characteristic discs of photoreceptor cells has been demonstrated. Natural mutations in the protein are unable to produce this effect, due to improper folding, processing or protein assembly.

Finally, the mechanism by which retinol is taken up by the visual system and how retinol binding protein dysfunction seriously perturb vision will be outlined.

S04. Lessons from rare disorders: The Bardet-Biedl syndrome

P. L. Beales:

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The Bardet-Biedl syndrome is an uncommon disorder, traditionally viewed as having a recessive pattern of inheritance. First described some 140 years ago, significant advances in our understanding

of its pathogenesis have recently come to light. In the early 1990s, heterogeneity became apparent, both hindering and informing progress in the field. Although, all eight mapped loci have been identified, they do not yet account for all cases (in fact around 55% of families do not have identified mutations). Segregation analyses of some BBS families have also identified a departure from Mendelian inheritance requiring us to view this and perhaps other disorders in a new manner. Most recently however, our studies have indicated that BBS is fundamentally a disorder of primary cilia function which might account for the retinal degeneration and renal tubular abnormalities characteristic of the syndrome. I will summarise the wealth of evidence that has brought us to these conclusions and highlight some of the recent discoveries within this rare disorder which continue to enlighten us.

S05. Nephronophthisis

C. Antignac;

Inserm U574 and Department of Genetics, Paris 5 University, Necker Hospital, Paris, France.

Nephronophthisis (NPHP) is an autosomal recessive, genetically heterogeneous, chronic nephropathy characterized by renal interstitial fibrosis and medullary cyst formation. It represents the most common genetic cause of end-stage renal disease (ESRD) in children and adolescent. Three distinct forms of the disease (juvenile, adolescent and infantile) have been described depending on the age of onset of ESRD. Extra-renal anomalies have been described in association with juvenile NPHP, namely retinal dystrophy [Senior-Løken syndrome (SLSN)] in 10-20% of the patients, ocular motor apraxia (Cogain syndrome), bone and liver anomalies.

Five genes have been identified so far, *NPHP1* and *NPHP4* in juvenile nephronophthisis, *NPHP3* in the adolescent form, *IQCB1/NPHP5* in SLSN and *INVS* encoding inversin in the infantile form. All except the inversin gene are novel genes. They encode nephrocystins, widely expressed cytosolic proteins containing multiple protein-interacting domains. They are all, at least partially, localized to the primary cilia in renal tubular cells, as virtually all products of human renal cystic genes. However, at least for inversin and nephrocystin-4, they also show various other sub-cellular localizations, which altogether suggests that nephrocystins are implicated in various cell processes, such as cytoskeleton organization, cell cycle regulation, adhesion processes and cilia function. Furthermore, nephrocystin-5 is expressed in the connecting cilia of photoreceptors and interacts with RPGR (retinitis pigmentosa GTPase regulator), thereby suggesting a common mechanism for retinal and kidney defects.

S06. Molecular basis of congenital nephrotic syndrome

M. Zenker;

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Congenital nephrotic syndrome (CNS) is a heterogeneous disorder caused by loss of glomerular permselectivity. It presents with proteinuria and edema at or even before birth. The common target of different pathogenetic mechanisms leading to CNS are the podocytes. These cells cover the outer surface of the glomerular capillaries and, by their interdigitating foot processes, form highly specialized intercellular contacts, the filtration slits representing the intrinsic protein barrier. CNS is mostly genetic. In the more common, **isolated** CNS, autosomally recessively inherited mutations in the genes encoding nephrin (*NPHS1*; OMIM 602716) and podocin (*NPHS2*; OMIM 604766), proteins expressed specifically at the slit diaphragm, can be frequently found. Certain mutations in the transcription factor *WT1* (OMIM 607102), which is specifically expressed in podocytes, are associated with early-onset nephrosis and due to diffuse mesangial sclerosis (DMS) and Denys-Drash syndrome in males (OMIM 194080). Still, the mechanisms of aberrant *WT1* signalling that lead to defective podocyte differentiation are incompletely understood. The genetic basis of other **syndromic** forms of CNS is even less known. We recently reported that loss-of-function mutations of *LAMB2* lead to Pierson syndrome (OMIM 609049) characterized by CNS with DMS and distinct ocular anomalies. Laminin β 2, a component of the glomerular basement membrane and certain other basal laminae, is believed to convey critical extracellular matrix signals for the differentiation and attachment of podocytes. Thereby, molecules at the cell-matrix interface and intracellular transducers

of matrix-derived signals came in the focus as potential candidates for other syndromic forms of CNS of which disorders associated with cerebral maldevelopment (e.g. Galloway-Mowat syndrome; OMIM 251300) represent an important subgroup. Additionally, animal models have implicated several genes (*neph1*, *itga3*, *actn4*, *cd2ap*; *arhgdia*) in the pathogenesis of nephrotic disorders. Future progress in that field will probably lead to a substitution of the conventional morphological classification of CNS by a new, etiology-based nosology.

S07. Mitochondrial dysfunction in neurodegeneration

A. Suomalainen-Wartiovaara, A. Hakonen, P. Luoma, S. Heiskanen, K. Peltonen, H. Tyynismaa;

Programme of Neurosciences and Department of Neurology, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland.

Mechanisms involved in mitochondrial DNA maintenance have recently turned out to be important causes of neurodegeneration. The proteins involved are nuclear-encoded, targeted to the mitochondria, and they participate in mtDNA replication and possibly repair. Their defects are often associated with secondary mutagenesis of mitochondrial DNA, or mtDNA depletion. Polymerase gamma, POLG, is the replicative DNA polymerase of the mitochondria. The mutations of its catalytic alpha subunit are the most common cause of familial progressive external ophthalmoplegia (PEO). However, recent data suggest that the clinical phenotypes resulting from POLG defects are exceptionally variant. We recently reported that dominantly transmitted parkinsonism can be caused by POLG mutations. The age-of-onset of the symptoms varied between families from early to late onset. A severe infantile multisystemic disorder, Alpers disease, was also found to be caused by recessive POLG mutations. Furthermore, we identified POLG spacer domain mutations underlying a spinocerebellar ataxia phenotype, which has turned out to be the most common adult ataxia-type in Finland, and possibly also in the Western and Northern Europe. Recessive mutations of the helicase-partner of POLG in the mtDNA replication, called Twinkle, also may result in a spinocerebellar ataxia disease, manifesting already in the early childhood. In the SCA group, these mitochondrial SCAs mostly resemble Friedreich's ataxia. The data of ours and colleagues indicate that mitochondrial DNA maintenance mechanisms are of crucial importance for the well-being of neurons, and their defects can result in late-onset neurodegeneration, as well as in acute infantile encephalopathies. Our recently developed transgenic mouse model may give clues to the pathogenetic mechanisms.

S08. The assembly of OXPHOS complexes in health and disease

L. Nijtmans;

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Mitochondria are considered to be 'the powerhouse' of the cell. In this organelle, the oxidative phosphorylation (OXPHOS) system confers the energy released by the breakdown of organic nutrients to produce ATP, the free energy currency of the cell. The mammalian OXPHOS system comprises five large membrane complexes, which are believed to be organised in higher order assemblies, supercomplexes. The complexes are built from numerous polypeptide subunits and prosthetic groups and their biosynthesis is a complicated process that requires both nuclear and mitochondrial gene products. Many genetic defects can occur that cause improper function of the OXPHOS system and a decrease in ATP production, but also can lead to an increase of toxic reactive oxygen species.

Although many mutations have been found to be associated with a large spectrum of clinical phenotypes, they cover only a fraction of the proteins involved in the biogenesis of mitochondria. Still a number of known and unknown OXPHOS defects remain to be explained at the genetic-molecular level. It is very likely that many new mutations in mitochondrial proteins will be detected in the future.

Our studies into the biogenesis of the OXPHOS system have revealed that defects can occur at various stages. In addition the use of functional complementation studies allowed the identification of a mutation in a component of the mitochondrial translation machinery causing progressive hepatoencephalopathy. This finding reveals a new class of proteins as a potential cause of mitochondrial disorders.

S09. Immunohistochemical tests for mitochondrial dysfunction**R. Capaldi;***Institute of Molecular Biology, University of Oregon, Eugene, OR, United States.
No abstract received.***S10. Genetic and Epigenetic Changes in Early Carcinogenesis****T. D. Tlsty;***Department of Pathology, UCSF Comprehensive Cancer Center, University of California at San Francisco, San Francisco, CA, United States.*

Studies of human epithelial cells and fibroblasts from healthy individuals are providing novel insights into how early epigenetic and genetic events affect genomic integrity and fuel carcinogenesis. Key epigenetic changes, such as the hypermethylation of the p16 promoter sequences, create a previously unappreciated pre-clonal phase of tumorigenesis in which a subpopulation of epithelial cells are positioned for progression to malignancy (Nature 409:636, 2001). These key changes precede the clonal outgrowth of pre-malignant lesions and occur frequently in healthy, disease-free individuals (Cancer Cell 5:263, 2004). Prior work from our laboratory has shown that surrounding stroma can dramatically influence tumorigenesis. Proper stromal-epithelial interactions can actually suppress the expression of preneoplastic phenotypes in epithelial cells and conversely, altered stromal-epithelial interactions can promote the probability that preneoplastic lesions progress to malignancy (Cancer Research 61:5002, 1999). Understanding more about these early events should provide novel molecular candidates for prevention and therapy of cancer.

S11. Cell Cycle Control: How to Preserve Genome Integrity during Cell Division ?**E. A. Nigg;***Max-Planck-Institute for Biochemistry, Dept. of Cell Biology, Martinsried, Germany.*

The error-free segregation of duplicated chromosomes during cell division is vital to the development and growth of all organisms. Chromosomal instability and imbalances (aneuploidy) are typical of many solid human tumors and often correlated with malignancy. Many chromosome aberrations are likely to result from the deregulation of mitotic progression, a defective spindle checkpoint and/or centrosome abnormalities. Our research aims at elucidating the role of protein kinases (and phosphatases) in the control of cell division (Nat Rev Mol Cell Biol. 2001, 2:21-32), the detailed function of the spindle assembly checkpoint (EMBO J. 2002, 21:1723-1732; Science 2002, 297:2267-7220), and the regulation of the centrosome cycle (Nat Rev Mol Cell Biol. 2001, 2:21-32). This talk will focus on the human centrosome, the major microtubule-organizing center. In every cell cycle, the centrosome needs to be duplicated once, and only once - much like the genome. We have been able to show that several protein kinases control distinct steps in the centrosome cycle (Dev Cell. 2003, 5:113-25). Furthermore, in collaboration with Matthias Mann's laboratory (Univ. of Southern Denmark, Odense), we have recently used a mass-spectrometry based proteomics approach to establish an inventory of centrosome components (Nature 2003, 426:570-574). Finally, we have begun to investigate the mechanisms that might cause centrosome amplification in human tumor cells. Our results identify both errors during mitosis and/or cytokinesis (EMBO J. 2002, 21:483-492) and *bona fide* overduplication of centrosomes (Mol Biol Cell (2005), 16:1095-1107) as prominent routes to numerical centrosome aberrations.

S12. Functional genomics of the Wnt signaling pathway in tumorigenesis**J. Behrens;***Nikolaus-Fiebiger-Zentrum, University Erlangen, Erlangen, Germany.*

Colorectal tumors develop as a consequence of genetic alterations in various tumor suppressor genes and oncogenes. The tumor suppressor APC (adenomatous polyposis coli) is mutated at an early stage of colorectal tumorigenesis and has been proposed to act as a gatekeeper in this process. Mutations of APC lead to aberrant activation of the Wnt signaling pathway by stabilization of the cytoplasmic component β -catenin and formation β -catenin/TCF transcription factor complexes. Thus, constitutive activation of β -catenin/TCF target genes including c-myc is a key event in the development of colorectal carcinomas and

also various other tumors, such as hepatocellular carcinomas. Wnt signaling is controlled by the negative regulator conductin (also termed axin2 or axil) or the related protein axin, which induce degradation of β -catenin by functional interaction with APC and the serine/threonine kinase GSK3 β . We show that conductin but not axin is a target of the Wnt signaling pathway and is upregulated in human colorectal and liver tumors as well as in the APC-deficient intestinal tumors of Min mice. Upregulation of conductin may constitute a negative feedback loop that controls Wnt signaling activity. Furthermore we present evidence that conductin is involved in mitotic checkpoint control and generation of chromosomal instability linking aberrant Wnt signaling to alterations of genomic integrity of cancer cells.

S13. VEGF in amyotrophic lateral sclerosis**P. Carmeliet;***Centre for Transgene Technology and Gene Therapy, KU Leuven, Campus Gasthuisberg, Leuven, Belgium.**No abstract received.***S14. Leukoencephalopathies: from MRI pattern to basic defect****M. van der Knaap;***Department of Child Neurology, Free University Medical Center, Amsterdam, The Netherlands.**No abstract received.***S15. BDNF Signaling in Anorexia and Bulimia****X. Estivill¹, M. Ribases¹, J. M. Mercader¹, H. Howard¹, B. Puchau¹, F. Fernandez-Aranda², M. Gratacos³;**

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Eating disorders (ED), such as anorexia nervosa (AN) and bulimia nervosa (BN), predominantly affect women and adolescents, and have a high prevalence and mortality rate in European populations. Although environmental factors play an essential role in the aetiology of ED, there are strong evidences of a genetic participation in the predisposition and development of AN and BN. Experiments on murine models and pharmacological studies in rats indicate the potential role of BDNF in the regulation of food intake and body weight as an anorexigenic factor. We have performed several case-control studies finding a consistent association between the -270C/T BDNF SNP and bulimia nervosa (BN), and the Val66Met variant to both AN and BN in six European populations. Furthermore, haplotype relative risk analysis and the transmission disequilibrium test (TDT) in ED trios for BDNF alleles have been confirmed in a large collection of trios from different European countries. We have analyzed 24 SNPs with a minor allele frequency higher than 0.10, covering the entire BDNF gene using the SNPlex technology in a total sample of 174 ED patients and 174 sex-matched unrelated controls, permitting a dense and targeted genetic characterization of the variability of BDNF in ED. We have also screened the entire NTRK2 gene in patients with ED and show evidence of association of a specific NTRK2 haplotype with binge-eating/purging AN, and a reduced frequency of another haplotype in BN patients. Finally, we have assessed BDNF plasma levels in 50 discordant sib pairs with ED and found that BDNF levels were significantly higher ($P = 0.004$) in ED patients than in their unaffected sibs. These data strongly indicate that this neurotrophic factor has an important role in the genetic susceptibility to ED and strongly argue for a role of BDNF signaling in eating behavior and body weight regulation.

S16. Digital karyotyping**M. R. Speicher;***Institut für Humangenetik, Technische Universität München, Munich, Germany.**No abstract received.*

S17. Canceromics: Molecular, cellular and clinical biochip technologies for cancer genetics

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Our aim has been to develop and apply new biochip technologies and approaches for high-throughput drug target and biomarker discovery, validation and therapy selection in oncology. This has involved development of microarray strategies in the molecular, cellular and clinical "dimensions".

Molecular biochips: We are applying microarray-based Comparative Genomic Hybridization (CGH) and Non-sense Mediated RNA Decay (NMD) to identify genes that undergo mutational activation or inactivation in cancer. CGH and NMD microarray data may highlight critical primary causative genetic events in the multi-step progression of cancer. As demonstrated by several recently approved targeted drugs for cancer, such mutated genes represent attractive targets for the development of effective cancer-specific therapeutics.

Cell-based biochips: We are developing cell-based functional microarrays for the high-throughput analysis of gene and protein functions in living cells. A number of different cell biochip platforms can be applied, including the reverse transfection approach (or "cell carpet" approach), where cDNAs, siRNAs or other biomolecules are printed on microscope slides and cells plated to grow on top of the array. Functional cell-based microarray studies make it possible to identify and validate drug targets in a highly parallel, miniaturized fashion, eventually in a genomic scale. Cell biochips can also be used for cell-based drug screening as well as for the analysis of causative gene-drug interactions. These technologies may in the near future facilitate the design of individualized therapies and more effective therapy combinations.

Clinical biochips: Sample-based microarray strategies, such as tumor tissue microarrays (TMA), facilitate the analysis of individual DNA, RNA and protein targets in thousands of arrayed patient samples, typically from formalin-fixed tumors. TMA analysis with target specific antibodies can rapidly establish definitive clinical diagnostic profiles for molecular targets, and quantify drug target distributions at the population level (target epidemiology). Further development of methods to print tissue lysates from frozen samples in an array format will help to automate and expand antibody-based screening of molecular targets in large cohorts of tissue specimens.

In summary, biochip technologies can be applied in molecular, cellular and clinical studies, thereby expanding the concept of "microarray analysis" beyond traditional gene expression studies. Integration of the various microarray platforms will facilitate a deeper, integrated, "systems biological" and mechanistic understanding of cancer cell biology, which eventually will be required for the development of next-generation targeted therapeutics for cancer.

S18. Single-molecule detection *in situ* using padlock and proximity probes

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Total genome sequence information provides a theoretical overview of all nucleic acids and protein molecules in cells and tissues. We now need efficient strategies to probe this multitude of molecules for information about their distribution, concentrations, interactions, and about their roles in biological processes.

We have established a set of molecular tools that represent detected DNA, RNA, or protein molecules as short, circular strings of DNA for highly specific analysis of large sets of molecules in solution, or for *in situ* analyses of even single or interacting sets of molecules.

Padlock probes are linear oligonucleotide probes that are converted to DNA circles in the presence of specific target nucleic acid sequences. Large sets of such circles can be amplified and identified, for example by hybridization to universal tag microarrays. The method presently permits genotyping of tens of thousands of DNA sequences in parallel.

In **proximity ligation**, antibodies are equipped with DNA strands

that can be joined by ligation when pairs of reagents bind the same target protein molecule. The process effectively reverse translates target proteins to linear or circular signature DNA molecules that can then be amplified and identified in extremely sensitive and precise homogenous or solid-phase protein assays. The procedure is being adapted for parallel analyses of large sets of proteins.

Both reacted padlock and proximity probes can be amplified by copying the circular molecules in so-called **rolling-circle replication** reactions. We have shown that the method allows analysis of the intracellular distribution of individual mitochondrial genomes, differing in single-nucleotide positions. In a variant of this procedure, proximity ligation-based detection of single or interacting pairs of Myc and Max proteins can be locally amplified by rolling-circle replication, for single molecule detection *in situ* in cells and tissues.

S19. *cis* regulatory variation in the human genome

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The goal of this work is to identify and characterize functionally variable regulatory regions that are likely to contribute to complex phenotypes and disorders in human populations, through effects on regulation of gene expression. We surveyed gene expression levels for ~ 700 genes in a sample of immortalized lymphoblastoid cell lines from 60 unrelated humans of the CEPH pedigrees, and used the publicly available HapMap SNP genotypes of the same individuals to perform association analyses, in an attempt to localize the genetic determinants of these quantitative traits. Approximately 300 of the 700 genes gave a detectable expression signal relative to background, and for most of those loci, we observed significant gene expression variation among individuals. We identified loci that exhibited highly significant associations between gene expression and SNP variants located *cis*- to the coding locus. We are finding many regulatory haplotypes several Mb away from the target gene suggesting that the regulatory landscape may be different from what has been hypothesized. For other genes, a *cis*- signal was detected, but its effect was spread over many SNPs and thus did not meet our significance threshold. By working with the complete genomic set of HapMap SNP genotypes, we were also able to identify significant *trans*-acting SNPs influencing expression variation.

S20. Activation of Non-coding RNAs by Epigenetic Therapy

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The abnormal methylation of CpG islands located near the transcriptional start sites of human genes plays a major role in carcinogenesis. Methylation of cytosine residues in these regions is associated with alterations in chromatin structure which reinforce gene silencing. Abnormally silenced genes serve as targets for epigenetic therapy in which the goal is to reverse DNA methylation and chromatin changes and reactivate silenced genes. Until now, the focus has mainly been on protein coding genes but we have now found that epigenetic therapy can activate microRNAs (miRNAs) which in turn can downregulate specific gene products by translational repression. Simultaneous treatment of human cancer cells with 5-aza-2'-deoxycytidine and phenylbutyric acid (epigenetic therapy) resulted in the activation of miR-127. Activation of miR-127 transcription was accompanied by changes in the DNA methylation and increased levels of histone H3 acetylation and lysine 4 methylation around the transcriptional start site. Interestingly, we also detected decreased levels in BCL6 protein, a potential target of miR-127. Thus, epigenetic therapy can activate expression of some microRNAs resulting in downregulation of target products important in human carcinogenesis.

S21. Ultraconserved elements in the human genome

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The availability of multiple mammalian genomes has allowed us to partially infer the evolutionary history of most of the bases in the human genome since the time of the mammalian radiation. Analysis has revealed about 500 DNA segments that show remarkably strong negative selection: stretches of at least 200bp that are totally unchanged in human mouse and rat, and averaging 96% identity with species as distant as chicken (diverged about 310 MYA). A significant number of these ultraconserved elements overlap the mRNAs of genes, especially genes involved in RNA processing. The remainder, which constitute the majority of the ultraconserved elements, neither code for protein nor appear in the UTRs of known genes. These latter elements do not appear to have any orthologs in invertebrate species. They often appear in clusters within ~1 Mb regions surrounding transcription factors involved in embryonic development. Experimental analysis of some of these elements has provided evidence that many are distal enhancers, located many hundreds of Kb from the transcription factor that they regulate. The most extremely conserved elements in the human genome (>1000bp, >99% identical in chicken) lie in the introns of POLA, the gene for DNA polymerase alpha on chromosome X. Recent evidence suggests that these ultraconserved elements do not regulate POLA, but rather are distal enhancers for a gene located a few hundred Kb downstream from them. This is the aristaless related homeobox (ARX) gene, involved in forebrain development, and associated with X-linked myoclonic epilepsy with spasticity and intellectual disability, X-linked West syndrome, Partington syndrome, nonsyndromic mental retardation, and X-linked lissencephaly with abnormal genitalia. We will discuss the evolutionary origins and properties of these and the other intriguing ultraconserved elements.

S22. Overview of preimplantation diagnosis

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Following its first clinical application in the year 1990, PGD (Preimplantation genetic diagnosis) was integrated into in vitro fertilization programs for the analysis of genetic disorders before the corresponding embryo is transferred to the patient. This approach represents an important alternative for couples at high reproductive risk which otherwise, in case of an affected fetus, have to decide whether to interrupt a pregnancy after conventional prenatal diagnosis. Throughout the years, the indications were expanded and now PGD is proposed in the following situations: 1) to carriers of monogenic diseases; 2) to carriers of balanced translocations; 3) to couples at risk of generating high proportions of aneuploid embryos for which they are exposed to failed or abnormal implantation; 4) to couples having an affected child requiring bone marrow transplantation from an HLA-identical sibling; and 5) to carriers of predisposition to late onset disorders.

According to the reports of the International Working Group on PGD and the ESHRE PGD Consortium, the demand for PGD has constantly increased and the number of cycles performed worldwide until now exceeds 6000. The reported pregnancy rate ranges from 25 and 30% per cycle with an incidence of obstetric and neonatal complications similar to that reported after ART. The incidence of malformations at birth is also in the same range as that characterized in ICSI children. The attitude towards PGD is extremely different in the different countries, due to cultural and religious aspects. This variability is present also in Europe, with some countries like Austria, Germany, Italy and Switzerland prohibiting by law the use of the technique. As a result, a form of reproductive tourism began, and couples questing for PGD are now looking for it in the European countries in whom PGD is currently applied.

S23. Circulating fetal cells and cell free fetal DNA: what is the current status?

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Fetal cells from maternal blood were first enriched in 1979 by Herzenberg and co-workers using the then newly developed approach of flow cytometry. In the intervening years developments in molecular biology (FISH and PCR) permitted the reliable analysis of such cells. In addition the development of more efficacious or simpler enrichment methods (MACS) lead to the remise that diagnostic applications would soon become a reality. These hopes have largely been dashed by the large scale NIH funded NIFTY study, in which close to 3000 clinical cases, enriched by either MACS or FACS and examined by XY-FISH, indicated that these approaches were not all suited for clinical use. Recent data has also suggested that the fetal erythroblast, may not be the most suitable target cell, as its nucleus displays an apoptotic character which renders it impervious to FISH analysis. For this reason current endeavours, are focussing on optimised enrichment procedures, automated detection, and the analysis of other cell types, such as trophoblasts. These analyses are largely being conducted in a multi-centre manner in the recently funded EU Network of Excellence "SAFE".

The presence of cell free circulatory nucleic acids was first reported almost 60 years ago by Mandel and Métais, however, it is only in recent years that this phenomenon has gained the interest of the wider scientific community, especially in oncology and prenatal medicine. Prompted by observations made in cancer patients, that tumour-derived DNA was detectable in plasma samples, Lo and colleagues in 1997 detected cell free fetal DNA in the plasma of pregnant women, thereby opening a new avenue for the non-invasive assessment of fetal genetic traits. In those instances where the fetal loci being interrogated are completely absent from the maternal genome, such as the fetal RhD gene in RhD pregnant women, this approach has been found to be so reliable that it is already being used clinically in several European centres.

The detection of other fetal genetic loci which differ slightly from maternal ones has proven to be more problematic, as the vast majority (>95%) of circulating cell free DNA in maternal plasma is of maternal origin. Therefore, the detection of fetal loci involving point mutations, which are of greatest interest for single gene disorders, has been restricted to single case reports.

In this regard we have recently made the observation that circulatory fetal DNA sequences are generally smaller than comparable maternal ones, and that by selecting sequences with a small size a selective enrichment of fetal DNA sequences can be attained. By using this approach we have shown that otherwise masked fetal loci, such as STRs or point mutations, can be reliably detected. By optimising this approach, it is hoped, that future developments will permit the detection of both the paternal and maternal fetal loci, thereby permitting a clear non-invasive prenatal diagnosis.

Once again, these facets are being explored in the NoE "SAFE".

S24. Practice of preimplantation genetic diagnosis

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No abstract received.

S25. LD in genetic isolated populations

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No abstract received.

S26. Selecting the right SNPs for genetic mapping studies in European populations

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The success of genetic mapping strategies for both rare and common disorders is critically dependent on the choice of study populations. As an increasing number of national genetic databases are being set up, sampling strategies have come under scrutiny. For the identification of genes mutated in Mendelian disorders, samples from isolated

founder populations especially from the periphery of the European subcontinent have proved extremely valuable mainly because of the uneven distribution of disease causing variants between and within populations and because of the wealth of genealogical data often available in isolates. Both simulation and experimental studies also suggest that the genetic variety provided by diverse populations will in the same way optimize the use of linkage disequilibrium mapping of common disease genes.

Because of the low effects observed in complex disorders, large sample sizes are required which can often not be collected in more isolate populations. This brings about an urgent need for the determination of genetically defined subpopulations within continental populations. An appropriate number of tagging SNPs has to be determined for each of those subpopulations to ensure sufficient capture of information especially in whole genome mapping approaches. Recent studies testing population based samples from across Europe suggest that the CEPH trios used by the HapMap Consortium as a reference for tagSNP selection will only provide partial information for an as-yet-unknown proportion of genes, and especially for peripheral European populations.

S27. Influence of LD on high-density SNP genome linkage scans

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No abstract received.

SP1. The success of the oppositions against the BRCA1 patents: how did it occur and what will be the impact on genetic testing?

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In 2001, the European Patent Organisation (EPO) has granted 3 patents on the BRCA1 gene. The patentees included Myriad Genetics, the United States of America and University of Utah. Their option to strictly exert their monopoly right by requesting that all diagnostic testing be done at Myriad Genetics Laboratories in Utah, has evoked strong reactions. Several European genetic societies and research institutes, and other parties including the Dutch and Belgian governments, have filed oppositions against these patents. These procedures have recently been concluded.

In May 2004, the first patent, which claimed 'a method for diagnosing a predisposition for breast and ovarian cancer' by comparing the patient's BRCA1 sequence with a reference sequence, has been revoked. In January 2005, the 2 other patents were maintained after the final hearings, but in amended - say slimmed - form: they no longer include a method for diagnosis, but only relate to a probe for the BRCA1 gene and a probe for the common Ashkenazi 185delAG mutation, respectively.

In practice, these patents will no longer interfere with diagnostic testing for familial breast and ovary cancer in Europe. The successful attack on the patents was based on errors contained in the original sequence, as it was disclosed in the patent application in 1994. On the discussion about 'inventive step' for diagnostic methods or specific mutations, the EPO has largely decided in favour of the patentees. The authors witnessed the hearings in Munich and would like to give the genetic community a firsthand account of the case.

LB1. The Human Telomeric maintenance Proteins trf2 and trf1 are involved in a EARLY DNA damage response

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Human cells protect their genomes by using DNA damage surveillance networks that can activate DNA repair, cell cycle checkpoints and apoptosis in response to <4 double-strand breaks (DSBs)/genome.

These same networks tolerate telomeres, in part because the TRF2 protein prevents recognition of telomeric ends as DSBs. Unexpectedly, we find TRF2 and another telomere maintenance protein, TRF1, involved in the DSB response.

TRF1 and TRF2 form transient foci that colocalize with laser microbeam-induced DSBs in human fibroblast nuclei. TRF1 and TRF2 are detectable at DSBs <3 seconds post-irradiation, earlier than ATM, a kinase that controls the major DSB surveillance network. Like many DNA damage response proteins, TRF2 is phosphorylated after DSB induction. Phospho-TRF2 associates with induced DSBs but not undamaged telomeres. The TRF2 response to DSBs is dependent on its basic domain and occurs in the absence of functional DNA-PKcs, the MRE11/Rad50/NBS1 complex or the Ku70, WRN and BLM repair proteins. Multiple lines of evidence suggest that TRF2 and ATM functionally interact at DSB sites: the two proteins colocalize at DSBs, γ -ray induced phosphorylation of TRF2 is ATM-dependent, and over-expression of TRF2 impairs DSB-induced ATM autophosphorylation as well as ATM-dependent phosphorylation of H2AX and p53.

These results provide evidence that TRF2 and TRF1 interact with DSB-containing chromatin, TRF2 competes with or attenuate ATM responses to DSBs and DSB-induced modifications may shift TRF2 from telomere maintenance to DSB repair. Our findings also indicate that human cells practice a strategic economy in using the same proteins in telomere maintenance and DSB repair.

LB2. Mutations in ESCO2, establishment of cohesion 1 homolog 2 (S. cerevisiae), cause Roberts syndrome

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Roberts syndrome (RBS; MIM 268300) is an extremely rare autosomal recessive disorder. Clinical characteristics include pre- and postnatal growth retardation, microcephaly, bilateral cleft lip and palate, mental retardation and tetraphocomelia. Cells from Roberts syndrome patients show a characteristic premature centromere separation, also known as heterochromatin repulsion/puffing, or the RS effect. To identify the disease locus we performed genome wide homozygosity mapping using a cluster of four RBS families from Colombia that shared a common ancestor in the XVIIIth century and an additional fifth Colombian family. Significant linkage was established for chromosome 8p12-21.2 between markers D8S258 and D8S505. Multipoint linkage analysis of 11 affected individuals gave a maximum LOD score of 13.4 at marker D8S1839. A new transcript LOC157570, recently renamed as ESCO2, appeared to encode a protein that belongs to a conserved family of proteins which play a role in the establishment of cohesion between sister chromatids. We performed sequence analysis of ESCO2 and identified homozygous mutations in all patients that were studied. In 19 patients from 16 families of different ethnic backgrounds eight different mutations were identified (one nonsense, one missense and six frameshift mutations). Northern analysis identified a predominant mRNA of approximately 3.3 kb in all human fetal tissues tested and in a subset of tested adult tissues (thymus, placenta and small intestine). We suggest that the RBS-related developmental abnormalities result from the impaired cell proliferation observed in RBS cells and that this growth impairment results from the cohesion defect and subsequent mitotic arrest.

LB3. Prospects of a Protein Therapy by means of PTDs-MeCP2 fusion protein for Rett syndrome

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Mutations in the MECP2 gene are the principle cause of the Rett syndrome and several lines of evidence show that the disease is caused by a loss of function of the MeCP2. It is therefore plausible that

the "introduction" of a wild type MeCP2 into the brain should be able at least to mild the course of the disease. Recently it has been reported that the protein transduction domain (TAT) when fused to a variety of proteins is able to deliver them into cells and even into the brain of living animals. We have produced large amount of recombinant MeCP2 isoform A protein tagged at the N-terminus with the TAT peptide. The data collected until now demonstrate that the TAT-MeCP2 protein is able to transduce after 8 to 10 hours in vitro several cell lines including primary fibroblasts of patients with Rett syndrome with an efficiency of 100%. The treatment of a clonal fibroblast cell line expressing the T158M mutation with the TAT-MeCP2 recombinant protein reverts the hyperacetylation of both H3 and H4K16 to a level comparable to the one of the cell line expressing the wild type MeCP2. The TAT-MeCP2 when injected intraperitoneally is able to trespass the Blood-Brain-Barrier and localizes in the nuclei of wild type mice. Experiments in knockout mice show that the TAT-MeCP2 protein influences the acetylation state of both H3 and H4K16.

Our results show that it is possible to introduce a consistent amount of biologically active recombinant proteins into the brain of knock out animals proving that the transduction technology may be applied to genetic diseases.

Acknowledgements

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C01. The splitting of human chromosome bands into sub-bands

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The splitting of chromosome bands into their sub-bands has its implications for the precise mapping of DNA probes at the sub-band level and for the understanding of the chromosome architecture. Surprisingly, there have been nearly no scientific investigations dealing with that process. Here we investigated the hierarchically organised splitting of bands in detail using the multicolour banding (MCB) probe set of different human chromosomes (#5, 6, 18, 19, X) hybridised to normal human metaphase and prometaphase chromosomes at the different band level. The analysis were performed by comparing the disappearance and appearance of pseudo-colour bands of the four different band levels according to ISCN95. The regions to split first are telomere- and centromere-near. In contrast to the GTG-band ideograms published in ISCN 95 at the 850-, 550-, and 400-band level pseudo-colours assigned to GTG-light bands are resistant to band splitting. GTG-dark bands split into their dark and light sub-bands because inside dark bands light sub-bands appear which are rather resistant to further elongation. This confirms the results obtained by stretching of GTG-banded chromosomes (Cytogenet Cell Genet 79:162-166, 1997). In this respect the nomenclature of the ideograms of GTG-banding patterns for normal human chromosomes should be reassessed. Furthermore, the results indicate to fundamental doubts on the well established concept of chromosome condensation during mitosis which should be replaced by the recently proposed concept of chromosome region-specific protein swelling.

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C02. Peculiarities of 5-methylcytosine-rich DNA distribution on adult and fetal human metaphase chromosomes

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DNA methylation is an epigenetic modification of human genome and is known to have an essential role in regulation of gene silencing and stabilization of chromosome structure.

We have investigated 5-methylcytosine-rich DNA localization on human metaphase chromosomes and have carried out a comparative analysis of 5-methylcytosine distribution on fetal and adult chromosomes from

PHA-stimulated lymphocytes. 72 metaphases from adult and 56 from fetal lymphocytes were analyzed. Immunofluorescent detection with monoclonal antibodies was performed for 5-methylcytosine-rich DNA regions revealing. Fluorescent signals appeared to be preferentially localized in the certain regions. Specific landmarks were identified for each chromosome pair, the new type of banding which we called M-banding was described. Intensity of signals varied. Pericentromeric heterochromatin of chromosomes 1 and 16, short arms of acrocentric chromosomes, majority of T and some R-bands demonstrated the most intensive fluorescence. Average to weak fluorescence was detected in majority of R-, in some T-bands and in pericentromeric heterochromatin of chromosomes 3 and 9. Very weak fluorescence was observed in all G-bands. Thus, the majority of GC-rich bands of 6, 9, 10, 13, 15 chromosomes (6q15, 6q21, 6q23, 9p13, 9q22, 9q32, 10q24, 13q22, 15q15, 15q24) demonstrated hypomethylated status, suggesting their special functional activity in lymphocytes.

Certain bands of fetal and adult chromosomes, with similar M-banding patterns, differed in their fluorescence intensity (chromosomes 1-3, 5-18, 20-22). Differences in staining of pericentromeric heterochromatin of chromosomes 1, 9, 16, heteromorphic staining of homologous chromosomes were registered.

Further analysis of structural and functional peculiarities contributing to M-banding characteristics in other adult and embryonic human tissues is needed.

C03. Systematic re-examination of carriers of balanced reciprocal translocations identifies multiple candidate regions for late-onset and common disorders

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Balanced reciprocal translocations associated with genetic disorders have facilitated the identification of genes especially for monogenic disorders with an early-onset. To assess whether chromosomal breakpoints may be associated with common and later onset disorders, we performed a systematic questionnaire-based re-examination of known reciprocal translocation carriers in Denmark.

In total, questionnaires were mailed to 875 carriers of which 733 accepted to participate (compliance = 84%). The reported traits/diseases were confirmed by medical files and personal contact with the family.

We observed 27 unrelated carriers with a disease where a breakpoint involved a cytogenetic band known to harbour a corresponding locus. This included very common disorders (e.g. allergy, asthma, myopia, obesity, hypertension, coronary heart disease, Type 2 diabetes), autoimmune disorders (Type 1 diabetes, inflammatory bowel disease, hyperthyroidism), neurological/neuropsychiatric disorders (e.g. bipolar disorder, multiple sclerosis, Parkinson's disease) and cancer. We found a significant linkage (LOD = 2.1) of dyslexia and a co-segregating translocation with a breakpoint in a confirmed locus for dyslexia, and we identified 10 other families, where the translocation co-segregated with a specific phenotype. Furthermore, we identified carriers with the same disease (e.g. cervix dysplasia, hypertension), where independent breakpoints involved the same chromosomal band.

Our study indicates that a systematic re-examination of translocation carriers promises to be a powerful way to identify loci and genes for a variety of human traits and disorders. To expand this approach to larger populations, we have initiated an international consortium to systematize the questionnaire-based re-examination and data-handling. For further information, see <http://www.mcndb.org>.

C04. Molecular karyotyping detects structural low grade mosaics in ~4 % of patients with idiopathic mental retardation and multiple congenital aberrations

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Chromosomal abnormalities are a major cause of mental retardation and multiple congenital anomalies. Screening for these chromosomal imbalances has mainly been performed in the past by standard or high resolution karyotyping. Unfortunately, the resolution of these analyses is limited to about ~5 to 10 Mb. Recently, so-called array or matrix CGH was developed to provide a better resolution for detection of subtle structural rearrangements. The resolution of array CGH depends on the size and density of the genomic fragments. Since arrayCGH provides a molecular approach for genome wide detection of unbalanced karyotypic defects, we propose the term *molecular karyotyping*.

In this study, molecular karyotyping was performed using arrays of 1 Mb resolution. Eighty patients with mental retardation, multiple congenital anomalies and normal standard karyotypes were analysed. In 18 patients (~22%) a chromosomal imbalance was detected and confirmed by FISH analysis. Interestingly, in three cases we found evidence for mosaicism for the detected structural chromosomal abnormality. Mosaicism ranged between 20 and 60%.

Our results indicate that in over 20% of patients with unexplained mental retardation and congenital anomalies cryptic chromosomal imbalances can be detected when using arrayCGH at 1 Mb resolution. We show that not only the resolution, but also the sensitivity to detect mosaics for small structural defects is higher than traditional karyotyping.

*These authors share equal contribution to the work presented in this abstract.

C05. CASP8 constitutional haploinsufficiency in a girl with del(2)(q32.3-q33.1) associated with congenital abdominal neuroblastoma, psychomotor retardation and dysmorphisms

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Neuroblastoma is a neuroblastic tumor of neuroectodermal cells derived from the neural crest. We studied a patient that underwent to medical evaluation at 2 and 4 years of age due to psychomotor and growth retardation, hypotonia, joint laxity and some dysmorphic features. She was operated soon after birth for a monolateral abdominal stage 1 neuroblastoma identified prenatally. No chemotherapy but only surveillance was recommended. Cytogenetic and FISH analysis revealed a complex translocation involving chromosomes 6, 10 and 15 with four breakpoints. The same translocation was found in her normal mother. The FISH breakpoints were narrowed by PCR amplification of STS markers in somatic hybrids. No cryptic deletions were present at the translocation breakpoints neither in the proposita nor in her mother. Later array-CGH experiments demonstrated a 2q32.3q33.1 deletion. FISH refined the deletion breakpoints within BAC RP11-267L23 and the two BACs RP11-53H10 (deleted) and RP11-35B12 (not deleted). The same region deleted in the patient (about 8 Mb) was inverted in one of the maternal chromosomes 2. Microsatellites analysis confirmed the maternal origin of the deletion.

The deleted 2q32.3q33.1 region contains genes known to be involved in neuroblastoma: CASP8 (and also CASP10), and caspase-8-related FLICE-inhibitory protein (FLIP). Usually CASP8 gene is deleted or silenced by methylation in neuroblastoma cell lines while methylation of its promoter region is the predominant mechanism in tumor samples. Constitutional hemizygous deletions involving these apoptotic genes have never been reported. DNA copy number changes and methylation studies in the tumor sample of the proposita are in progress.

C06. Interphase FISH mapping of translocation breakpoints using paraffin-embedded tissue: Identification of a candidate gene for phocomelia

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Disease-associated balanced chromosomal rearrangements (DBCRs) have proved to be extremely useful in mapping disease loci and the positional cloning of disease-causing genes. In these cases the phenotype is likely to arise from direct interruption of one or more genes or regulatory elements. Generally, breakpoint mapping is done using fluorescent in-situ hybridisation (FISH) on metaphase preparations but these are not always available. We have extended our previously reported technique for mapping DBCR breakpoints using interphase FISH to enable the use of archive paraffin embedded tissue sections. We have shown that this is an effective strategy using two cases with *de novo*, apparently balanced translocations: namely: t(1;2)(q41;p25.3) with bilateral renal dysplasia and t(2;12)(p25.1;q23.3) with upper limb phocomelia and lower limb phocomelia. The latter case has proven particularly interesting as the gene CMKLR1 was found at the translocation breakpoint on chromosome 12. This gene is known to be expressed in the developing limb and is likely to be part of the retinoic acid signalling pathway. Our own studies using immunocytochemistry suggest that the limb expression may be myoblast specific. The phenotype of the proband is consistent with recent data suggesting that myoblasts may be required for normal limb outgrowth. We are currently screening a case with a similar phenotype but no visible chromosomal abnormalities for mutations in CMKLR1 and a mouse model is being created. This gene is an excellent candidate for the limb phenotype and its discovery emphasises the usefulness of this method in studying cases with no available metaphase chromosomes.

C07. Rad50 deficiency causes a variant form of Nijmegen Breakage Syndrome

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The Mre11/Rad50/Nbs1 complex is assumed to act as a sensor of DNA double-strand breaks. Mutations of Mre11 and Nbs1 are associated with the radiation sensitivity syndromes ataxia-telangiectasia-like disorder (ATLD) and Nijmegen breakage syndrome (NBS), respectively. Here we report that Rad50 deficiency also occurs as an inherited condition in man due to hypomorphic *RAD50* germline mutations. An 18-year-old German who has a variant form of the NBS without immunodeficiency, is a compound heterozygote for a nonsense and a stop codon mutation in the *RAD50* gene. Rad50 protein expression is reduced to less than one tenth in her fibroblasts and lymphoblastoid cells. This Rad50 deficiency is associated with a high frequency of spontaneous chromatin exchanges. Rad50 deficient fibroblasts fail to form Mre11 and Nbs1 foci in response to irradiation. Phosphorylation of p53 at serine 15 and the transcriptional induction of p21/WAF1 mRNA are reduced and Ser343 phosphorylation of Nbs1 is undetectable in the patient's lymphoblastoid cell line following irradiation. These defects could be complemented by transient transfection of wildtype Rad50 cDNA into the lymphoblastoid cells. Our findings expand the clinical spectrum of DNA double strand break repair disorders and provide evidence that Rad50 is required for the recruitment of Mre11 to sites of DNA damage and modulates some functions of the ATM kinase.

C08. Expression analysis of murine hemochromatosis genes in anemia and iron overload

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Hereditary Haemochromatosis (HH) is an autosomal recessive disorder common among Caucasians, characterized by iron homeostasis disruption leading to parenchymal iron overload. Five different disease forms have been characterized and the involved genes (HFE, HJV, HEPC, TFR2 and SLC40A1) have been individuated. All these genes are highly expressed in the liver and the correspondent iron proteins

(HFE, hemojuvelin, hepcidin, Tfr2 and ferroportin1) seem to be correlated. Nevertheless, the molecular details of their interrelationship are still unclear. To clarify *in vivo* iron proteins function we utilized murine models of two different strains in different iron conditions. Here we report the liver expression of hepcidin, ferroportin and of the other HH genes after phlebotomy-induced anaemia and following parenteral and oral iron loading. Furthermore, TFR2 and ferroportin proteins expression variations have been investigated in liver and duodenum. Results demonstrate in liver a statistically significant reduction of hepcidin and ferroportin expression after phlebotomy, an increase of Hepc and a decrease of Tfr2 expression after iron loading. Hepatic ferroportin protein is decreased during anemia, while, 6 hour after phlebotomy, when hepatic hepcidin expression is increased, SLC40A1 duodenal expression increases but ferroportin resulted to be diminished. This data would confirm that ferroportin protein is regulated by hepcidin post-traditionally.

In parenteral iron overload condition, TFR2 expression and protein production decrease, while HFE expression remains constant. On the contrary, in dietary iron overload TFR2 remains constant while HFE expression increases. This data supports the hypothesis that Hfe and Tfr2 are part of different pathways both causing final hepcidin activation.

C09. Gain-of-function amino acid substitutions drive positive selection of FGFR2 mutations in human spermatogonia and explain the high prevalence of *de novo* Apert syndrome mutations.

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Despite the importance of mutation in genetics, there are virtually no experimental data on the occurrence of specific nucleotide substitutions in human gametes. We have studied the occurrence of spontaneous mutations at position 755 of Fibroblast Growth Factor Receptor 2 (FGFR2) because 755C>G transversions cause Apert syndrome; this mutation, encoding the gain-of-function substitution Ser252Trp (birth prevalence: 1:100,000), occurs with a birth rate elevated 500-800 fold above background and originates exclusively from the unaffected father. We previously demonstrated high levels of both 755C>G and 755C>T FGFR2 mutations in human sperm, and proposed that these particular mutations are enriched because the encoded proteins confer a selective advantage to spermatogonial cells (Goriely *et al.*, *Science* **301**, 643; 2003).

We have now obtained further evidence consistent with this proposal. First, we show that mutation levels at the adjacent FGFR2 nucleotides 752-754 are low, excluding any general increase in local mutation rate. Second, we present three instances of double nucleotide changes involving 755C, expected to be extremely rare as chance events. Two are shown, either by assessment of the pedigree or by direct analysis of sperm, to have arisen in sequential steps; the third (encoding Ser252Tyr) was predicted from structural considerations. Finally we demonstrate that both major alternative spliceforms of FGFR2 (*Fgfr2b* and *Fgfr2c*) are expressed in rat spermatogonial stem cell lines.

Taken together, these observations show that specific pathogenic FGFR2 mutations attain high levels in sperm because they encode proteins with gain-of-function properties, favoring clonal expansion of mutant spermatogonial cells in the human testis.

C10. A 12 gene DNA resequencing chip for molecular diagnosis of hypertrophic cardiomyopathy

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease with a prevalence of approximately 1/500 adults. To date, mutations

in 14 genes responsible for familial HCM have been identified. The majority of these mutations are single nucleotide substitutions and have been identified throughout coding exons, intron-exon junctions and in promoter regions. Genetic testing for HCM is of considerable benefit in diagnosis, prognosis or predictive testing. However, the genetic and allelic heterogeneity of HCM means that mutation detection by classical methods is time-consuming, expensive and difficult to realise in a routine diagnostic molecular laboratory. We have developed a 30 kb CustomSeq Resequencing Array (Affymetrix) enabling rapid molecular diagnosis of HCM. This array comprises all coding exons (161), splice-site junctions and known promoter regions of 12 genes mutated in HCM. We designed PCR probes for a total of 44 amplicons (size ranging from 330-10'800 bp) which are pooled and hybridised to the array. Using this array, more than 90% of all mutations reported to date can be detected by a single hybridisation experiment.

We report here the results of a first series of unrelated patients with HCM that we have analysed by our CustomSeq array and discuss our experience with this new highthroughput molecular diagnostic tool.

C11. An FGF23 missense mutation causes familial tumoral calcinosis with hyperphosphatemia

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Familial tumoral calcinosis (FTC) is an autosomal recessive disorder characterized by ectopic calcifications and elevated serum phosphate levels. Recently, biallelic deleterious mutations in the *GALNT3* gene have been described to cause FTC. We investigated a 12-year-old boy from Austria with FTC who was negative for *GALNT3* mutations. He clinically presented with painful swellings at the left elbow and left tibia. Radiographs showed a calcified tumoral mass at the left elbow and signs of diaphysitis at the left tibia. The FTC phenotype is regarded as the metabolic mirror image of hypophosphatemic conditions, where causal mutations are known in the genes *FGF23* or *PHEX*. Hence, we considered these genes candidates for underlying FTC. Sequencing revealed a homozygous missense mutation in the *FGF23* gene (p.S71G) at an amino acid position which is highly conserved. Wild-type FGF23 is secreted as intact protein and processed N-terminal and C-terminal fragments. Expression of the mutated protein in HEK293 cells showed that only the C-terminal fragment is secreted whereas the intact protein is retained in the Golgi complex. Also, determination of circulating FGF23 in the affected individual with an FGF23 ELISA showed a marked increase in the C-terminal fragment. These results suggest that the FGF23 function is decreased by absent or extremely reduced secretion of intact FGF23. We conclude that FGF23 mutations in hypophosphatemic rickets and FTC have opposite effects on phosphate homeostasis.

C12. Homozygosity for a Dominant-Negative Type I Collagen Mutation Attenuates the Type IV OI Phenotype of the Heterozygous Brtl Mouse: Insight into Disease Mechanism

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The Brtl mouse is a dominant-negative model for type IV osteogenesis imperfecta, caused by a glycine substitution (G349C) knocked into one *col1a1* allele. Brtl/+ pups have 30% perinatal lethality. Surviving Brtl mice are small with weaker and more brittle bones.

In murine models for other dominant genetic disorders, homozygous animals have a more severe or lethal phenotype than heterozygous.. We present the novel genetic situation in which homozygosity for a dominant mutation (Brtl/Brtl) attenuates the phenotype of the heterozygous mice. Brtl/Brtl have normal perinatal survival, an intermediate weight versus Wt and Brtl/+, and lack the rib fractures, flared thorax, osteoporotic calvarium and vertebrae seen in Brtl/+. Brtl/Brtl femurs have normal BMD, but intermediate CSA, TbTh and BV/TV. They withstand normal loading to fracture and are less brittle than Brtl/+. Cell numbers, MAR and BFR/BS were unchanged in all

genotypes at 2 mos.

Matrix insufficiency and collagen chain composition may contribute to the different homozygous and heterozygous phenotype. In Brtl/+ type I collagen containing one mutant chain is selectively retained by the cells and is deficient in lung and skin tissue. Approximately 1/3 of collagen with one mutant chain and 2/3 of collagen with two mutant $\alpha 1$ chains are secreted from the Brtl/+ fibroblasts, resulting in about 40% matrix insufficiency of type I collagen in Brtl/+ versus only 15% in Brtl/Bratl. Additionally, collagen with one mutant chain present in Brtl/+ has a reactive SH group which might contribute to illegitimate collagen cross-links, while collagen of Brtl/Bratl contains only disulfide-linked $\alpha 1$ dimers.

C13. Germ-line and somatic PTPN11 mutations in human disease

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We recently demonstrated that germ-line mutations in *PTPN11* cause Noonan syndrome (NS), and that somatic mutations in the same gene recur in a heterogeneous group of hematologic malignancies. *PTPN11* encodes the protein tyrosine phosphatase SHP-2, a transducer that relays signals from activated receptors to RAS and other intracellular signaling molecules. Both germ-line and somatic *PTPN11* mutations promote SHP-2 gain-of-function by destabilizing the catalytically inactive conformation of the protein.

Here, we describe the diversity of *PTPN11* mutations in human disease, and trace the parental origin of *de novo* *PTPN11* lesions in NS.

DNAs from individuals with NS (N=107), juvenile myelomonocytic leukemia (N=64), myelodysplastic syndromes (N=234), chronic myelomonocytic leukemia (N=84) and acute myeloid leukemia (N=393) were screened by DHPLC analysis. Mutations were identified in 49 subjects with NS and 45 individuals with a hematologic disorder. Most of mutations altered residues located in or around the interacting surfaces of the N-SH2 and PTP domains that stabilize SHP-2 in its catalytically inactive conformation. However, no overlap in the precise residue substitutions was observed. Such dramatic genotype-phenotype relationship defines a novel class of mutations of *PTPN11* with role in cancer. These data also support that distinct gain-of-function thresholds for SHP-2 activity are required to induce cell-, tissue- or developmental-specific phenotypes, each depending on the transduction network involved.

Finally, by analyzing intronic portions flanking the exonic *PTPN11* lesions in 49 sporadic NS cases, we traced the parental origin of mutations in 14 families, and demonstrated that they were transmitted by the father in all cases.

C14. The Loeys-Dietz syndrome: a new aortic aneurysm syndrome caused by mutations in TGFBR1 and TGFBR2

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A novel aortic aneurysm syndrome characterized by the triad of widely spaced eyes (hypertelorism), bifid uvula and/or cleft palate, and generalized arterial tortuosity with ascending aortic aneurysm/dissection was previously described (Loeys-Dietz syndrome; ASHG 2004). This syndrome shows autosomal dominant inheritance and variable clinical expression. Here we present other findings in multiple systems that include: craniosynostosis, Arnold-Chiari malformation with hydrocephalus, mental retardation, congenital heart disease, and aneurysms with dissection throughout the arterial tree. While some individuals show some overlap with Marfan syndrome (MFS)

(variable evidence for bone overgrowth and aortic root dilatation and/or dissection), none satisfied diagnostic criteria for MFS. Importantly, aneurysms appeared to be particularly aggressive, with rupture at a very early age or at a size below that seen in MFS.

We identified heterozygous mutations in either type I or type II transforming growth factor β receptor (T β RI or T β RII) in 4 and 6 families, respectively. Despite evidence that receptors derived from selected mutant alleles cannot support TGF β signal propagation, heterozygous patient-derived cells do not show altered kinetics of the acute phase response to administered ligand. Furthermore, tissues derived from affected individuals show increased expression of both collagen and connective tissue growth factor (CTGF), as well as nuclear enrichment of phosphorylated Smad2, indicative of increased TGF β signaling. These data definitively implicate perturbation of TGF β signaling in many common human phenotypes including craniosynostosis, cleft palate, arterial aneurysms, congenital heart disease, and mental retardation, and suggest that comprehensive mechanistic insight will require consideration of both primary and compensatory events.

C15. LMX1B genotype-renal phenotype studies in 32 families with nail patella syndrome

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Nail-patella syndrome (NPS) is characterized by developmental defects of dorsal limb structures, nephropathy, and glaucoma and caused by heterozygous mutations in the LIM homeodomain transcription factor *LMX1B*. In order to identify possible genotype-phenotype correlations, we performed *LMX1B* mutation analysis and in-depth investigations of limb, renal, ocular, and audiological findings in 106 subjects from 32 NPS families. Remarkable phenotypic heterogeneity at the individual, intrafamilial, and interfamilial level was observed for different NPS manifestations. We detected low tension glaucoma (LTG) and hearing impairment as new symptoms associated with NPS. Quantitative urinalysis revealed proteinuria in 21.3% of individuals. Microalbuminuria was observed in 21.7% of subjects without overt proteinuria. Interestingly, nephropathy appeared significantly more frequent in females. A significant association was identified between the presence of renal involvement in an NPS patient and a positive family history of nephropathy. Sequencing of *LMX1B* revealed 18 different mutations, including 9 novel variants, in 28 families. Individuals with an *LMX1B* mutation located in the homeodomain showed significantly more frequent and higher values of proteinuria compared to individuals carrying mutations in the LIM-A and LIM-B domains. No clear genotype-phenotype association was apparent for extrarenal manifestations. This is the first study indicating that family history of nephropathy and mutation location might be important in precipitating the individual risk for developing NPS renal disease. We suggest that the NPS phenotype is broader than previously described and that LTG and hearing impairment are part of the syndrome. Further studies on modifying factors are needed to understand the mechanisms underlying phenotypic heterogeneity.

C16. A new XLMR syndrome characterized by mental retardation, primary ciliary dyskinesia and macrocephaly, caused by a novel mutation in OFD1

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We report on a new syndrome characterized by mental retardation, primary ciliary dyskinesia (PCD) and macrocephaly in a large three-

generation family with an X-linked recessive inheritance. The 11-year-old index case had delayed motor development, severe mental retardation (IQ=20), and macrocephaly. Recurrent respiratory problems led to the diagnosis of PCD at the age of 8 years. Eight other affected males died because of bronchopulmonary infections before the age of 5 years. Five obligate female carriers were clinically inconspicuous. We performed pairwise and multipoint linkage analysis using SNP markers, and found tight linkage to a 25 cm interval in Xp22.32-Xp21.3 (LOD score 2.99 at DDX8019). Based on the specific phenotype of PCD, the candidate interval was screened for cilium-associated genes. Therefore, we performed an *in silico* comparative genomic approach, comparing 164 genes from the candidate interval with those from *Chlamydomonas reinhardtii* as a ciliated and those from *Arabidopsis thaliana* as a non-ciliated organism. For 4 of these genes, orthologs were only detected in *C. reinhardtii*. Subsequent screening of these genes revealed a causative mutation in the *OFD1* gene, known to be mutated in X-linked dominant Oral-Facial-Digital syndrome type 1 (MIM#311200). An insertion of four nucleotides (AAGA) in exon 16, present in affected males and all obligate carrier females, causes a frameshift leading to a premature stop codon. Thus, despite its different mode of inheritance, the disorder in this family is allelic to OFD1, and our findings suggest that the *OFD1* gene plays an important role in the biogenesis and/or functioning of cilia.

C17. CHARGE syndrome: the phenotypic spectrum of mutations in the *CHD7* gene

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The main features of CHARGE syndrome are coloboma, heart malformations, choanal atresia, retarded growth and development, genital hypoplasia, ear anomalies and/or deafness. Other common aspects of this syndrome are cleft lip/palate, balance disturbances, facial palsy and renal malformations. The clinical variability is high and a clinical diagnosis is not always straightforward.

Recently, the *CHD7* gene, a new member of the chromodomain gene family, was identified as an important gene involved in CHARGE syndrome¹. Since this discovery we studied more than 60 patients with CHARGE syndrome and identified *CHD7* mutations in over 60% of cases.

The clinical details of our *CHD7* positive patients confirm the phenotypic variability of CHARGE syndrome. This spectrum includes patients who do not meet the diagnostic criteria for CHARGE syndrome as proposed by Blake et al.², as will be illustrated by some atypical cases. For example a mutation was found in a boy with an above average IQ. Also in a patient without coloboma, choanal atresia or heart defect a mutation was identified. Furthermore we were able to demonstrate intrafamilial variability in sibs carrying the same *CHD7* mutation.

An overview of the mutations identified thus far will be given and the clinical spectrum of *CHD7* positive patients will be discussed.

1) Vissers LE. Nat Genet. 2004 Sep;36(9):955-7.

2) Blake KD. Clin Pediatr. 1998 Mar;37(3):159-73.

C18. Identification of a novel locus for Hirschsprung disease associated with microcephaly, mental retardation and polymicrogyria

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At least two syndromes present with a similar association of Hirschsprung disease, microcephaly and mental retardation, namely Mowat-Wilson syndrome (MWS) and Goldberg-Shprintzen syndrome (GOSHS). Although some of the key features in GOSHS are more or less similar to the ones in MWS, patients can be distinguished on the basis of their facial dysmorphism. Furthermore, MWS is associated with

de novo mutations in *ZFHX1B* located at 2q22, whereas for GOSHS, which is autosomal recessive inherited, no causative gene has been identified yet. To unravel the genetic basis of GOSHS we studied a large inbred Moroccan family segregating GOSHS and polymicrogyria as part of the syndrome. We identified, by homozygosity mapping, a critical region of 3.8 Mb on 10q21.3-q22.1. We sequenced all the genes in the region and identified a homozygous stop codon in a gene we named GOSHS. In a second Pakistani family we identified another homozygous stop codon. As mutations in this gene are associated with Hirschsprung disease and polymicrogyria, the encoded protein likely plays a role in neural crest and cerebral development.

C19. Different mechanism of second hit in neurofibromas depending on the germline *NF1* mutation

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Neurofibromatosis type I is an autosomal dominant disorder caused by mutations in the *NF1* tumor suppressor gene. The hallmarks of *NF1* are neurofibromas, café-au-lait spots, Lisch noduli and learning difficulties. The *NF1* population can, on the basis of their germline mutation, be subdivided into two main patient groups i.e. those with a mutation confined to *NF1* and those with a microdeletion of the entire *NF1* region (1.4Mb) including neighboring genes. Patients with an *NF1* microdeletion have on average a larger tumor load and have an increased risk for malignancy.

We investigated if the difference in tumor phenotype originates from a difference in somatic inactivation of *NF1*. We analysed 35 neurofibromas of *NF1* microdeletion patients and 28 neurofibromas of *NF1* non-microdeletion patients for LOH. LOH was found in 7/28 neurofibromas of the non-microdeletion patients and in none of the 35 microdeletion patients (P=0.002, Fisher exact). Using semi-quantitated PCR and array-CGH we further investigated the mechanism of LOH. Of the 7 neurofibromas with LOH, 3 exhibited LOH due to a deletion on chromosome 17, and 4 due to mitotic recombination.

The reason for the absence of LOH in neurofibromas of *NF1* microdeletion patients remains speculative. LOH in microdeletion cases results in a homozygous deletion of 17 genes and this might not be viable for the Schwann cell or alternatively this might lead to a malignant tumor. This study points to an important difference in the pathogenesis of neurofibroma formation in both *NF1* patient groups.

C20. Inducible oncogenic ERBB2 signaling identifies premature senescence as a primary tumor-protective response in mammary carcinogenesis

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ERBB2 plays a central role in the development of breast cancer and other epithelial malignancies. Enhanced ERBB2 expression leading to aberrant signaling in the course of tumorigenesis can frequently be attributed to genomic amplification of the ERBB2-locus on chromosome 17q21. In order to inducibly express oncogenic ERBB2 (NeuT) in MCF-7 breast carcinoma cells we applied a tetracycline (Tet)-controlled expression system. Interestingly, tet-mediated upregulation of NeuT does not result in a mitogenic response but instead leads to prominent biochemical and phenotypical alterations compatible with premature senescence (e.g. cell cycle arrest and expression of senescence associated beta-galactosidase). Molecular dissection of the underlying mechanisms provides direct evidence that upregulation of the cyclin-dependent kinase inhibitor P21 via the P38 MAPK pathway is necessary to elicit the senescence response upon oncogenic ERBB2 signaling.

Premature senescence represents a tumor-protective program that has previously been shown to be induced by overexpression of other oncogenes (e.g. activated forms of RAS or RAF). Our results suggest a multistep model of ERBB2-positive mammary carcinomas that anticipates further specific hits in addition to upregulation of ERBB2.

Such lesions may functionally inactivate premature senescence as the inherent anticarcinogenic program, targeting constituents of the premature senescence program (like P21), regulatory molecules, or downstream effectors.

C21. Pharmacogenetics studies in patients with advanced lung cancer: prognostic value of the thymidylate synthase 2R/3R polymorphism and predictivity of NER factor XPD K751Q and XRCC1 R399Q polymorphisms following platinum-based chemotherapy

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Lung cancer is one of the most frequent killers in most populations, and treatment is mainly based on platinum derivatives. Survival is limited and highly variable, probably depending both on tumor features and sensitivity to treatment. Pharmacogenetic studies can contribute to better understand both kind of variables. As candidate genes we choose TS, whose expression controls cell proliferation and the 3R allele is associated with higher expression, XPD, whose K751Q SNP affects repair activity, and the XRCC1 R399Q SNP. We enrolled 322 consecutive patients, 80% males, 86% smokers, 82% NSCLC. Among them, 244 received platinum derivatives, mainly in combination with gemcitabine. The control group was represented by 253 healthy medical students. Uni- and multivariate statistical analysis was performed with the SPlus package. On December 2004, the median follow up period was 320 days. Survival was estimated on a subgroup of 128 patients with enrollment <6 months from diagnosis and >9 months follow-up. Patients' genotype frequencies were not significantly different from controls'. Median survival was significantly increased in TS 2R homozygotes (17.3m) vs. 9.6m in 2R/3R and 8.7m in 3R/3R, independently of the treatment. The effect of platinum treatment was statistically significant in KK homozygotes, who showed a significantly longer survival (13.3m in Pt-treated vs 7.7m in nonPt-treated), while it was ineffective in QQ homozygotes. Relapses were 22.8% in KK and 43.5% in QQ. The XRCC1 SNP was not significantly associated with any of the clinical parameters.

Pharmacogenetics may predict non-responsiveness and address patients to alternative non-platinum based treatments.

C22. All-trans retinoic acid treatment of Wilms tumor cells reverses expression of genes associated with high risk and relapse in vivo

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¹Physiological Chemistry I, University of Wuerzburg, Germany, ²Institute of Molecular Biology and Tumor Research (IMT), University of Marburg, Germany. Wilms tumor is one of the most frequent neoplasias in children. Our previous microarray screening in a large series of Wilms tumors revealed several candidate genes that are deregulated in advanced tumors and are part of the retinoic acid signalling pathway. To investigate whether retinoic acid could be employed as a novel therapeutic agent in these tumors, we treated cultured Wilms tumor cells with different concentrations of all-trans retinoic acid (ATRA) and assessed gene expression changes by real time RT-PCR as well as microarray analysis. Several genes like RARRES3, CTGF, CKS2, CCNA2, IGFBP3, CCL2 or ITM2B that were previously found to be deregulated in advanced tumors exhibited opposite expression changes after ATRA treatment. In addition to enhanced retinoid signalling, the TGF β pathway was strongly activated by ATRA treatment of Wilms tumor cells. Both the retinoic acid and the TGF β pathway mediate inhibition of cell growth. These findings represent the first molecular evidence of a potential benefit from ATRA treatment in Wilms tumors.

C23. Follow-up Extension Study of a Double-Blind Phase 3 Clinical Study of Recombinant Human Arylsulfatase B (rhASB) in Patients with Mucopolysaccharidosis VI (MPS VI)

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MPS VI (Maroteaux-Lamy syndrome) is a lysosomal storage disease caused by insufficient activity of the enzyme *N*-acetylgalactosamine 4-sulfatase (arylsulfatase B or ASB). The clinical course is chronic, progressive, and life threatening. Results are reported here for a multicenter, multinational, open-label extension of the Phase 3 study of enzyme-replacement therapy (ERT) using weekly treatment of 1 mg/kg recombinant human arylsulfatase B (rhASB). Nineteen patients in each of the rhASB and placebo groups completing the 24-week placebo-controlled study were enrolled. The primary objective was to evaluate rhASB's ability to enhance endurance based on an increase in the number of meters (m) walked in a 12-MinuteWalk at Week 48 compared to baseline (Week 0) and with entry into the extension study (Week 24). As a reference, the rhASB group improved by a mean distance of 109 m relative to 26 m for the placebo during the double-blind phase ($p=0.025$). From Week 24 to Week 48, the original placebo group received rhASB and showed a mean increase of 65 m relative to Week 24 values ($p=0.007$), and an increase of 90 m relative to baseline for the entire 48 weeks ($p<0.001$). From Week 24 to Week 48, the original rhASB group improved their mean walk distance an additional 36 m ($p = 0.15$) for a total of 145 m relative to baseline ($p=0.001$). Similar changes were observed for each group in a 3-Minute Stair Climb, a secondary endpoint variable, providing additional evidence of improved endurance. Additional efficacy and safety data will be presented.

C24. Development of antisense-induced exon skipping for clinical applications in Duchenne Muscular Dystrophy.

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Manipulation of pre-mRNA splicing using antisense oligoribonucleotides (AONs) has shown great therapeutic promise for Duchenne muscular dystrophy (DMD). By inducing the skipping of one or more exons, an out-of-frame transcript associated with DMD can be converted into an in-frame transcript as found in patients with the less severe Becker muscular dystrophy (BMD). This may alleviate or even stop the progression of muscle wasting in DMD. We have characterized a series of AONs with which the skipping of 30 out of 79 DMD exons can be induced. Ten were successfully applied to cultured muscle cells from a series of DMD patients carrying a variety of mutations. The synthesis of BMD-like dystrophins was detected in up to 90% of treated muscle cells. Furthermore, by applying combinations of AONs, we demonstrated the simultaneous skipping of two, and even multiple, consecutive exons. This multixon skipping increases the therapeutic applicability to over 85% of DMD patients, and renders it significantly less mutation-specific. In the *mdx* mouse model, intramuscular injections of an exon 23 skipping AON restored dystrophin in up to 20% of fibers. We also set up human sequence-specific exon skipping in transgenic mice carrying a copy of the full-length human DMD gene. Intramuscular delivery of human-sequence specific AONs showed dose-and time-dependent skipping of the human (but not the murine) exons in hDMD mice. We are currently planning a clinical proof of concept study to determine the tolerability and efficiency of an exon 51-specific AON following local intramuscular injections in patients.

C25. Significant decrease of BMI in individuals carrying the 103I MC4R allele - Association analysis in 7937 participants of two population-based surveys

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The melanocortin-4-receptor gene (*MC4R*) is part of the melanocortinergic pathway that controls energy homeostasis. The *MC4R*_V103I (rs2229616) polymorphism was recently shown to be associated with body weight regulation: A meta-analysis of 14 case-control studies reported a mild negative association with obesity (OR=0.69, p=0.03). However, evidence in a large population-based study in a homogeneous population and a significant estimate of the change in quantitative measures of obesity was still lacking.

We performed an association analysis of the genotyping data of two population-based surveys of Caucasians with the same high quality study protocol including a total of 7937 participants.

Linear regression analysis showed a significant decrease of 0.52 BMI units (95%CI=[-0.02, -1.03], p=0.043) for carriers of the heterozygote rs2229616G/A genotype, a genotype observed in 3.7% of the participants. Logistic regression illustrated a significantly negative association of the *MC4R* variant with "above average weight" (BMI \geq median BMI) yielding an odds ratio of 0.75 (95%CI=[0.59, 0.95], p=0.017). Comparing obese (BMI \geq 30 kg/m², WHO 1997) to non-obese (BMI <30 kg/m²) we obtained similar results. The findings were detected for each gender and each survey separately and did not depend on the modelling of age-, sex- and survey effects.

Our study confirms previous findings of a meta-analysis that the relatively infrequent G/A genotype of the V103I_ *MC4R* polymorphism is negatively associated with obesity in population-based original data of 7937 participants. Our study extends previous findings by showing for the first time a significant decrease of BMI in individuals carrying the infrequent allele of this *MC4R* variant.

C26. Linkage of chromosome 7p and association of the GPRA gene region in Italian asthmatic families

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In 2001, a genome scan for asthma and IgE described linkage of a 20cM region on chromosome 7p14-p15 in Finnish and French- Canadian families, and in 2004 the GPRA gene region has been associated with elevated IgE in the same populations.

We performed the screening of chromosome 7 to investigate whether it may contain a susceptibility gene for asthma phenotypes in the Italian population. The screening of 117 families with 19 microsatellite markers showed potential linkage at different positions ranging from 22 cM to 54 cM in the marker map, for asthma (p<0.005 at 44 cM), elevated IgE (p<0.002 at 22 cM), and atopy (p<0.005 at 54 cM).

The PDT (pedigree disequilibrium test) was performed on 211 families using 7 SNPs in the GPRA gene region. Elevated IgE levels were associated with 2 SNPs (Hopo 546333 p=0.0046; Hopo 49 p=0.006) and with 7-SNP haplotypes in the global test (p=0.019).

These results are in agreement with the recent report in Finnish and French-Canadian families and suggest that a common susceptibility factor for atopic manifestations in asthma is likely to map in the GPRA gene region.

C27. Effect of *Dnmt1* mutations on transmission ratio distortion.

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Transmission-ratio distortion (TRD) (transmission of parental alleles that deviate from expected 1:1 ratio) has been observed in different species and at different genetic loci. We hypothesized that TRD resulted from failure to erase epigenetic marks during gametogenesis, and therefore TRD is one of the manifestations of epigenetic inheritance. This hypothesis was strongly supported by our finding of grandparental-origin dependent TRD for loci closely linked to imprinted regions in humans and mice. We found evidence for genetic heterogeneity with regard to TRD in human families. In the mouse, we determined that the preferential transmission of grandmaternal alleles (58%) in the imprinted region of chromosome 12 was due to

postimplantation loss of female embryos that inherited the alleles of their grandfather. To further investigate the mechanism underlying TRD and the role of methylation we repeated the backcrosses with mice that carry mutations in the DNA methyltransferase gene 1 (*Dnmt1*), which encodes the enzyme involved in maintenance DNA methylation and is essential for normal embryo development. Two mutations were tested: the *Dnmt1^c* and *Dnmt1ⁿ* that target the catalytic and N-terminal regions of DNMT1, respectively. As predicted by our model, *Dnmt1* mutations caused loss of TRD (P=0.0007), i.e. normal Mendelian transmission of alleles. Furthermore, the transmission ratios did not depend upon the genotype of the offspring, but depended upon the genotype of the mother, suggesting a maternal factor effect. These results confirm the role of methylation and *Dnmt1* in the genesis of TRD and explain the genetic heterogeneity observed in human studies.

C28. Identification of a fifth locus involved in Autosomal Dominant Hypercholesterolemia

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Autosomal Dominant Hypercholesterolemia (ADH), a major risk factor for atherosclerosis, is associated with mutations in two genes : *LDLR* (encoding low-density lipoprotein receptor) or *APOB* (encoding apolipoprotein B). Our team has pioneered the claim that the disease is far more heterogeneous. We have shown that defects in at least 2 other genes (*HCHOLA3* and *HCHOLA4*) are implicated in the disease and recently identified *HCHOLA3* as *PCSK9* (proprotein convertase subtilisin/kexin type 9). Through the ADH French Research Network, we collected genetic material from a large french pedigree. Molecular diagnostic laboratory excluded linkage to the major genes *LDLR* and *APOB* and sequencing analysis did not reveal any mutation in the *PCSK9* gene. Furthermore, the study of 6 microsatellite markers spanning the *HCHOLA4* interval clearly excluded linkage to this locus. To evaluate the power of the family for linkage, 500 simulations were carried out using the SLINK software in which genotypes were simulated using parameters compatible with ADH. The average lod score was 2.08 and the maximum lod score was 3.96 indicating that the statistically significant threshold of 3 can be reached in this single family. Genomewide scan will be performed in collaboration with the National Genotyping Center. After the fine mapping of the locus, candidate gene approach through sequencing and Southern blot will be developed to identify the corresponding gene. Identification of this gene, called *HCHOLA5*, could reveal a new regulatory pathway or mechanism and lead to the development of new potent drugs.

C29. Two new putative loci for ADNFLE identified in an Italian family suggest a digenic inheritance for the disease

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an idiopathic partial epilepsy characterised by cluster of short repeated seizures occurring mostly during non-REM sleep. This epileptic phenotype has been up to now considered a simple Mendelian trait caused by mutations in neuronal nicotinic acetylcholine receptor (nAChR) subunit genes. However, for the 88% of the identified cases such mutations were not identified. We recently demonstrated that in a three generation Italian family the disease was unlinked to all known ADNFLE loci as well as to all known brain-expressed nAChR subunits. We therefore performed a genome-wide linkage analysis on this family, by analysing approximately 400 STR markers equally distributed on the whole genome and calculating LOD and NPL scores. Two new putative ADNFLE loci (NPL score > 3) on chromosomes 3p22-p24 and 8q11.2-q21.1 were identified. These findings, together with several previously ADNFLE characteristics (i.e. the impossibility to draw a phenotype-genotype correlation, the variability of symptoms observed even among individuals of the same family and with the same genetic defect, the rarity of the identified mutations, the difficulty in linking the phenotype to the mutated receptor properties and the presence of

locus heterogeneity) which cannot be explained by a simple Mendelian inheritance of the disease suggest that this epilepsy could be, at least in the above family, a complex disorder. In particular, the reported data are conceivable with a digenic transmission of the disease.

C30. Co-localization on human chromosome 1 of susceptibility loci for Atopic Dermatitis (ATOD2) and Psoriasis (PSORS4)

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Atopic dermatitis (ATOD) [OMIM#603165] is a chronic inflammatory skin disease typified by itchy inflamed skin characterized by an onset mainly in early childhood. ATOD is a complex disease triggered by both genetic and environmental risk factors and twin studies indicate that the genetic contribution is substantial. Many genome wide linkage studies mapped a number of susceptibility regions on chromosomes 1q21 (ATOD2), 3q21 (ATOD1), 5q31-q33 (ATOD6), 13q12-q14 (ATOD5), 17q25 (ATOD4), 20p (ATOD3). Four of these loci (1q21, 3q21, 17q25 and 20p) are closely coincident with psoriasis [OMIM*177900] susceptibility loci, although ATOD is quite distinct from psoriasis and rarely the two diseases occur together in the same patient.

We performed a fine-mapping approach to refine the localization of ATOD2 and PSORS4 in a large series of 115 Italian nuclear families with ATOD and 128 trios with psoriasis using a set of 16 microsatellites mapping within 1q21 segment spanning 3.5 Mb. Genotype and haplotype analysis revealed that ATOD and psoriasis overlap in an interval of 65 Kb. This region contain at least 4 genes including SPRR2C, SPRR2G, LOR, PGLYRP3 and some ESTs. These results confirm preliminary reports on the existence of an ATOD susceptibility locus on human chromosome 1 and provide the exact localization in a smallest region of overlap (SRO) with PSORS4. This data support the "common soil" hypothesis (identical predisposing genes are involved in different complex diseases like diabetes, asthma, osteoporosis and inflammatory bowel disease).

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C31. Detection of large-scale copy number polymorphisms in the human genome

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Array-based comparative genomic hybridization (array-CGH) experiments, using 1 megabase interval microarrays containing Bacterial Artificial Chromosome (BAC)-based DNA clones, were performed in pair-wise analyses using genomic DNAs from 39 unrelated and apparently healthy individuals. These experiments led to the identification of over 200 loci in the human genome that contain large-size copy number polymorphisms, scattered throughout the human genome and account for locus-specific variations, some of which involve hundreds of kilobases of DNA. An average of 12 large-size polymorphisms were detected in a given individual when a pooled DNA source containing 10 unrelated, healthy individuals was used as a control DNA source. Over 50% of these genomic regions overlap with known genes and approximately 25% of the identified loci map to regions previously thought to contain segmental duplications. Interestingly, some 10% of the loci reside within 100 kb of gaps in the current presentation of the human genome. Together, these large-sized copy number variations may represent as much as a ten fold increase in human genetic variation than single nucleotide polymorphisms (SNPs). We have established a searchable database

that will provide an updated catalog of these large-size variations for accurate interpretation of whole-genome-directed array-based CGH assays in the research and clinical settings. This previously unappreciated large-scale genomic heterogeneity argues for a more dynamic impression of the structure of human genome. Further studies will likely yield evidence for whether these regions are associated with disease-associated rearrangements or account for genetic differences in susceptibility to diseases or reaction to specific environmental stimuli.

C32. Identification of submicroscopic DNA alterations in mental retardation using whole genome tiling-resolution arrayCGH

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Background: Mental retardation occurs in 2-3% of the general population and is caused by microscopically visible chromosomal aberrations in approximately 10% of the cases. The frequency of submicroscopic chromosomal alterations in these patients is, as yet, unknown. Array-based comparative genomic hybridization (arrayCGH) allows for the detection of submicroscopic chromosome alterations and, through this technology, the complete human genome can now be analysed at a 100 kb resolution.

Methods and patients: In total 100 patients with mental retardation and/or congenital anomalies were included in this study. Prior routine cytogenetics, including subtelomeric analysis, failed to reveal anomalies in all cases.

We constructed a 32K tiling resolution BAC array with complete coverage of the entire human genome. DNA from all patients were hybridized in duplicate onto this array, and novel statistical algorithms were developed for automated copy number analysis. Predicted alterations were validated by FISH and MLPA, and parents were tested for *de novo* occurrence.

Results: Tiling resolution arrayCGH screening detected DNA copy number alterations in most patients. Analysis of parental samples, however, indicated that the majority of these alterations were inherited large scale copy number variations, i.e., polymorphisms. Fourteen patients exhibited *de novo* alterations, 10 deletions and 4 duplications. These clinically relevant alterations varied in size between 300 kb and 10 Mb, and were scattered throughout the genome.

Conclusions: This study demonstrates the diagnostic value of tiling resolution DNA copy number screening in patients with mental retardation and congenital abnormalities, revealing causative submicroscopic alterations in ~14% of the cases.

C33. Interchromosomal segmental duplications of the pericentromeric region on the human Y chromosome

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Basic medical research critically depends on the finished human genome sequence. Two types of gaps are known to exist in the human genome: those associated with heterochromatic sequences and those embedded within euchromatin. We have identified and analysed a euchromatic island within the pericentromeric repeats of the human Y chromosome. This 450 kb island, although not recalcitrant to subcloning and present in 100 tested males from different ethnic origin, was not detected and is not contained within the published Y chromosomal sequence. The entire 450 kb interval is almost completely duplicated and consists predominantly of interchromosomal rather than intrachromosomal duplication events that are usually prevalent on the Y chromosome. We defined the modular structure of this interval and detected a total of 128 underlying pairwise alignments ($\geq 90\%$ and $\geq 1\text{ kb}$ in length) to various autosomal pericentromeric and ancestral pericentromeric regions. We also analyzed the putative gene content of this region by a combination of *in silico* gene prediction and paralogy analysis. We can show that even in this exceptionally duplicated region of the Y chromosome eight putative genes with open reading frames reside, including fusion transcripts formed by the splicing of

exons from two different duplication modules as well as members of the homeobox gene family DUX.

C34. Natural antisense transcripts (NATs) associated with genes involved in eye development

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Natural antisense transcripts (NATs) are a class of genes whose role in controlling gene expression is becoming more and more relevant. We describe the identification of eight novel mouse NATs associated with transcription factors (Pax6, Pax2, Six3, Six6, Otx2, Crx, Rax and Vax2) that play an important role in eye development and function. These newly-identified NATs overlap with the mature processed mRNAs or with the primary unprocessed transcript of their corresponding sense genes, are predicted to represent either protein coding or noncoding RNAs and undergo extensive alternative splicing. Expression studies, by both RT-PCR and RNA in situ hybridization, demonstrate that most of these NATs, similarly to their sense counterparts, display a specific or predominant expression in the retina, particularly at postnatal stages. We found a significant reduction of the expression levels of one of these NATs, Vax2OS (Vax2 opposite strand) in a mouse mutant carrying the inactivation of Vax2, the corresponding sense gene. In addition, we overexpressed another NAT, CrxOS, in mouse adult retina using adeno-associated viral vectors and we observed a significant decrease in the expression levels of the corresponding sense gene, Crx. These results suggest that these transcripts are functionally related to their sense counterparts and may play an important role in regulating the molecular mechanisms that underlie eye development and function in both physiological and pathological conditions.

C35. Dissection of gene regulatory networks in liver cells using chromatin immunoprecipitation and high resolution genomic arrays of the ENCODE regions.

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Detailed information on how genes in the human genome are regulated is not available. One objective for the ENCODE project is to evaluate methods to analyse this in 1% or 30 Mb of the genome. Antisera against HNF3b, HNF4a, USF1 and acetylated histone H3 were used in chromatin immunoprecipitation to enrich DNA interacting with each protein in the liver cell line HepG2. Precipitated DNA and reference DNA were differentially labelled and hybridised to a high resolution tiling path genomic array of ~19500 PCR fragments covering 75% of the ENCODE region. Spots with highly significant signal for the enriched DNA were identified. The results indicate that HNF3b and HNF4a are frequent regulators of gene activity in HepG2 cells, with 117 and 132 binding sites respectively. USF1 is more specialised with 29 binding sites. Many sites are in 5' ends of genes but presumed regulatory sequences are frequently also found in intronic and intergenic regions. Acetylated histones are mostly found at 5' ends of genes but also in other locations. Protein distribution was also determined in tissue microarrays comprising 45 different normal tissues, 20 cancers and 50 cell-lines. HNF4a is mutated in type 2 diabetes (MODY1) and together with HNF3b important for fatty acid metabolism. USF1 is proposed as the cause of familial combined hyperlipidemia so the results are of importance for common human diseases. This strategy can be used for many other transcription factors and with improvements in array technology may be scaled up to still larger parts of the genome.

C36. Identification of miRNA on chromosome 21.

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The 21-22 nucleotides long microRNA (miRNA) are an abundant class of non-coding RNA, that regulate gene expression. Hundreds of miRNA genes have been reported, and many of these were shown to be phylogenetically conserved.

To identify novel miRNA, we aligned the HSA21 genomic sequence with the syntenic mouse sequences and selected sequences between 36 and 99 bp long conserved with more than 70% identity and no gap. Note that more stringent criteria, i.e. sequences longer than 100bp, more than 70% identity and no gap were previously shown to be unsuitable to identify miRNAs, but perfect for Conserved Non-Genic sequences and coding-exons. We isolated a total of 2796 of these sequences and kept the seven candidates with the following features that characterize miRNAs: a) folding energies below -32.5 kcal/mol; b) 1 to 4 nt bulges and mismatches; c) GC content between 32.8% and 62.5%; and d) evolutionary conserved secondary structures.

To experimentally verify that these sequences were encoding bona fide miRNAs we tested their expression by RNase Protection Assay in total RNA extracted from 3 different cell lines. This procedure allowed to readily show that at least three of the candidates sequences hybridized to ~21-24 nt RNA. Further we analyzed their function with the DIANA-microT program (<http://www.diana.pcgi.upenn.edu>). We searched 18,000 UTR's of human genes and found more than 10 targets being conserved in human and mouse.

Our study provides novel insights into the identification of human miRNAs. We are currently investigating their target genes in order to validate their functionality.

C37. Whole Genome Genotyping (WGG) on High Density DNA BeadArrays.

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High resolution genotyping is a fundamental requirement for linkage disequilibrium-based disease or pharmacogenomic association studies. Even with the judicious selection of tagging SNPs by the International HapMap Project, at least several hundred thousand SNPs will need to be scored requiring a highly multiplexed genotyping assay that allows the user to select SNPs of interest. Standard PCR-based genotyping assays typically do not scale effectively and complexity reduction approaches cannot target specific SNPs.

As such, we have developed a novel, array-based whole genome genotyping (WGG) assay that overcomes the multiplexing barrier and effectively enables unlimited SNP genotyping from a single sample preparation. This was accomplished by hybridizing the product of a single-tube whole genome amplification (WGA) reaction to 50-mer probe arrays and conducting an array-based allele-specific primer extension assay. This approach is unique among genotyping methods in that essentially the entire genome is present in the sample; there is no need for a complexity reduction step. With this approach, multiplexing is only limited only by the number of features on the array.

We have combined this WGG assay with our high density BeadChips which currently support over 250,000 bead types (corresponding to 125,000 SNP assays) to create a BeadChip with over 100,000 "exon-centric" SNP assays. Processing of these BeadChips including hybridization, washing, array-based primer extension, and signal amplification is performed on an automated slide processing robot --- the Tecan Genesis GenePaint system. Details and performance of the 100k chip will be presented.

C38. Disentangling linkage disequilibrium and linkage from dense trio SNP data

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Parent-offspring trios are widely collected as part of disease gene mapping studies, and also being extensively genotyped as part of the International HapMap Project [1]. Transmission of haplotypes from parents to offspring is dependent on both linkage disequilibrium (LD) and linkage. With dense maps of markers on parent-offspring trios, the effects of LD and linkage can be separated allowing estimation of

recombination rates in a model-free setting. We define a model-free multipoint method based on dense sequence polymorphism data from parent-offspring trios to estimate recombination rates between adjacent markers. We present simulations to show that this method can detect recombination hotspots (>25 times background intensity) over a region typed with as few as 10 markers ($p<0.001$) and that the numbers of markers required to locate a hotspot of a given intensity decreases with marker density. Using Centre d'Etude du Polymorphisme Humain pedigree data across a 10Mb region of chromosome 20, a comparison of population recombination rate estimates obtained from our method with estimates obtained using a coalescent based approximate-likelihood method implemented in PHASE 2.0 [2] shows detection of same coldspots and most hotspots. Spearman rank correlation between the two sets of estimates is 0.58 ($p<2.2 \times 10^{-16}$).

[1] The International HapMap Consortium *et al.* [The International HapMap Project]. *Nature* 2003;426:789-796.

[2] Stephens M, Donnelly P *et al.* [A comparison of Bayesian methods for haplotype reconstruction from population genotype data]. *AJHG* 2003;73:1162-1169.

C39. A 2-locus TDT for testing gene-gene interaction: Application to the study of HLA-DRB1 and CTLA4 in Multiple Sclerosis

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Given the increasing interest in gene-gene interactions in the research of genetic factors in complex diseases, it is important to have reliable methods to assess these interactions. Here, we propose a straightforward method: the 2-locus TDT which considers the segregation of alleles for two independent genes conditionally to the parental genotypes at each locus. This approach may be used not only to test the effect of each gene but also their interaction. We develop this method to reconsider the suggested interaction between the HLA-DRB1 and CTLA4 genes involved in the predisposition to multiple sclerosis (Alizadeh *et al.*, *J Neuroimmunol*, 2003).

The typing of 1266 members of 422 French family trios (one affected patient and his two parents) is available for HLA-DRB1 gene and the -651 SNP (C/G) located in the promoter region of the CTLA4 gene. The effect of each gene is confirmed by applying the 2-locus TDT but without evidence of any interaction between the two genes. In contrast, when the transmission rate of the CTLA4 C allele from heterozygous parents is compared between the HLA-DRB1*15 positive and negative patients (homogeneity transmission test), we show a significant difference and we conclude to an interaction.

To explain this discordance, we study the robustness of the two approaches to population stratification. We show that the 2-locus TDT is robust against population stratification unlike the homogeneity transmission test. This latter test, which is often used in the literature, may thus lead to a spurious conclusion of interaction.

C40. Multivariate linkage analysis of specific language impairment

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Specific language impairment (SLI) is defined as a difficulty in developing language skills normally despite adequate intelligence and opportunity. Previous studies within the SLI Consortium have involved a full genome scan of 98 families affected with the disorder (wave 1), resulting in the identification of two possible quantitative trait loci (QTL) on chromosomes 16q (SLI1) and 19q (SLI2), and a replication of the two linkage regions using an additional 86 affected families (wave 2). All linkage results were obtained by applying Haseman-Elston and variance-components approaches univariately. Investigations have suggested that in certain situations multivariate analysis of traits would offer more power to detect linkage. This study therefore uses the multivariate variance-components approach on the original genome scan data to detect further QTL, and also to investigate the previously determined SLI1 and SLI2 using the combined sample of

waves 1 and 2. The multivariate genome scan highlighted three new possible QTLs on chromosomes 4, 5 and 10 with p -values < 0.003 . The multivariate method also allowed us to investigate the relationship between phenotypes influenced by loci SLI1 and SLI2.

C41. Chromosome 6p22 risk haplotype for dyslexia is associated with a reduced expression of KIAA0319 gene

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Dyslexia is one of the most prevalent childhood cognitive disorders and is caused in large part by genetic factors. We have recently identified an association with a haplotype on chromosome 6p22 and dyslexia (Francks *et al.*, 2004). The haplotype spans a 77 kb region of strong inter-marker linkage disequilibrium, encompassing the first four exons of KIAA0319, the entire TTRAP gene and the first exon of THEM2. Mutation screening by DHPLC of all exons and predicted promoters did not detect obvious functional variants that would disrupt any of the three genes. The risk haplotype might influence gene transcription regulation. To test this hypothesis we used the MassARRAY (Sequenom) platform to determine relative differences quantitatively in allele-specific transcription in cell lines that were heterozygous for the risk haplotype. We identified six lymphoblastoid and three neuroblastoma cell lines, which carry one copy of the risk haplotype and were heterozygous for at least one marker within the transcript of each of the three genes. Heterozygous markers in proximity of the promoters were also analysed in immuno-precipitated chromatin (haploChIP assay) from the lymphoblastoid cells. All markers analysed for TTRAP and THEM2 showed no quantitative differences between transcripts generated either on the risk or non-risk haplotypes. Conversely, all the markers used for KIAA0319 showed consistently a transcription reduction of about 40% associated to the risk haplotype. These data show, for the first time, a link between a genetic background and a biological mechanism that might be involved in the development of dyslexia.

C42. The molecular basis of autoimmunity: using celiac disease as a model to unravel common pathogenic pathways using a functional genomics approach

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Celiac disease (CD) is the most common food-induced (gluten) autoimmune disorder (AID) in the western world. CD patients have an increased risk of developing other AIDs, including type 1 diabetes, autoimmune thyroiditis and rheumatoid arthritis, implying a common genetic origin to AIDs. AIDs affect more than 5% of the population and are often associated with specific HLA alleles. CD is the best understood HLA disorder at the moment. However, HLA can only explain part of the genetic susceptibility. Notably, genetic studies indicate that certain non-HLA loci appear to predispose to multiple AIDs, suggesting common pathogenic mechanisms. Moreover, CD is characterized by alterations in intestinal mucosa permeability and these are also observed in other AIDs, suggesting a common pathogenetic role for an impaired integrity of the intestinal barrier at the onset of AIDs.

Using a sibpair approach we recently identified new CD loci on 6q, 9p and 19p. The 6q locus might represent a more common autoimmune locus. Large-scale SNP studies using Illumina technology are currently used in a cohort of 480 case/control pairs to fine-map these three regions. Using genetic-profiling on intestinal biopsies (the site of disease lesion) of CD patients revealed major disturbances in the cell differentiation/proliferation ratio. A more comprehensive study determined the effects of gluten withdrawal on the restoration of the intestinal mucosa and has led to novel insights into the role of the enterocytes and the epithelial barrier in CD.

This combined genetic/genomic approach will help to increase our knowledge on AID pathogenesis.

C43. Interstitial deletion of chromosome 9q22.32-q22.33: a novel cause of syndromic overgrowth

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Overgrowth syndromes are characterized by a height over + 2 SD in association with a variable combination of macrocephaly, mental retardation, facial dysmorphic features, advanced bone aging and hemihyperplasia. Apart from well known conditions and chromosome anomalies - such as Sotos syndrome or del(22)(q13.1q13.2) - a number of overgrowth patients remain undiagnosed.

In the course of a systematic screening of patients with unexplained overgrowth syndrome using microarray-based CGH, we identified two children with congenital overgrowth and nearly identical *de novo* interstitial deletions del(9)(q22.32-q22.33). The clinical manifestations include macrocephaly, pre and postnatal overgrowth (> + 2.5 SD), and distinctive facial features (frontal bossing, down-slanted palpebral fissures, epicanthal folds, long tubular nose, small triangular mouth with thin upper lip, long philtrum, low-set ears). They both have a psychomotor delay (walk at 24 months and a few words at 4 in case 1 and no walk and no speech at 33 months in case 2) and a strabismus. In addition, one child has a craniostenosis and the other a thyroglossal cyst.

Further analyses, based on the development of DNA microarrays covering the whole breakpoint intervals with fosmids and small-insert-clones (1.5 to 4 kb), allowed us to delineate the deletion breakpoints within 5-kb intervals and demonstrated that, although similar, the 6-Mb segment deleted in each patient correspond to different deletion boundaries.

These data suggest giving consideration to cryptic 9q22 deletions in the diagnosis of unexplained overgrowth syndromes and may help in understanding the mechanisms and genome architecture underlying chromosomal rearrangement in the 9q22.3 region.

C44. Pulmonary function abnormalities in children with osteogenesis imperfecta correlate with OI type and location of collagen mutation.

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Osteogenesis Imperfecta (OI) is characterized by osteoporosis and bone fragility. OI patients with severe chest wall deformities and scoliosis develop respiratory insufficiency. To determine whether early pulmonary abnormalities are detectable in children with types III and IV OI and to learn whether these abnormalities correlated with OI type, severity of scoliosis or location of type I collagen mutations, we designed a retrospective cross-sectional clinical study. Forty-seven children with OI types III and IV, age 4.9 to 23 years underwent 131 pulmonary function evaluations (PFT) over 7 years. In 39 children, the type I collagen mutation was identified. Multiple Regression analysis of FVC, TLC, VC (% of predicted), age, mutation, OI type and degree of scoliosis were performed. Our results reveal a PFT decline during childhood in both types of OI, (TLC: $r=-0.41$, $p<0.0001$; VC: $r=-0.48$, $p<0.0001$; FVC: $r=-0.45$, $p<0.0001$). Children with progressive type III OI had greater loss of FVC than moderate type IV OI. The decline of PFT with age was significantly greater in a2(I) than in a1(I) collagen chain mutations. PFT abnormalities correlated with severity of scoliosis. However a significant decline also occurred in children with minimal scoliosis. These findings suggest a direct effect of the abnormal collagen in lung tissue, (TLC: $r=-0.42$, $p=0.01$; VC: $r=-0.31$, $p=0.0005$; FVC: $r=-0.43$, $p=0.008$). Pulmonary abnormalities are detectable in asymptomatic children with types III and IV OI. This data provides the basis for careful early monitoring and therapeutic intervention in OI children to prevent cor pulmonare in adulthood.

C45. Brain malformations in oculocerebrocutaneous syndrome (OCCS)

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Oculocerebrocutaneous syndrome (OCCS), also known as Delleman-Oorthuys syndrome, is a rare multiple congenital anomalies/mental retardation syndrome characterized by a triad of eye, skin and brain malformations. The ocular features consist of orbital cysts and an-microphthalmia, and skin abnormalities comprise focal a-/hypoplastic skin defects and skin appendages. These features are well described. The neuropathological abnormalities however, have not been well delineated. Up to now, 28 patients have been reported, with a preponderance of males; recurrence has not yet occurred. The cause of OCCS is still unknown.

The brain imaging studies and further clinical data of 2 new and 9 previously reported patients could be (re)evaluated. We found a remarkably consistent pattern of malformations in eight of 11 patients, consisting of polymicrogyria, enlarged lateral ventricles or hydrocephalus, agenesis of the corpus callosum sometimes associated with interhemispheric cysts, and a novel mid-hindbrain malformation. The latter consists of a giant and dysplastic tectum, absent vermis, small cerebellar hemispheres in most cases, and a large posterior fossa fluid collection. We hypothesize that this mid-hindbrain malformation is pathognomonic for OCCS.

In particular the unique mid-/hindbrain malformation distinguishes OCCS from related syndromes with comparable forebrain anomalies (as Aicardi syndrome) and from syndromes with similar skin and eye features (as encephalocriocutaneous lipomatosis, oculo-auricular-vertebral spectrum, and focal dermal hypoplasia). The described pattern of malformation thus shows that OCCS is a separate entity and helps in differentiating it from other entities. The mid-hindbrain malformation points to a defect of the mid-hindbrain organizer as the underlying pathogenetic mechanism.

C46. Carbohydrate-deficient glycoprotein syndrome type Ia : clinical expression in 19 patients older than 12 years

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Whereas the paediatric symptomatology in CDG Ia syndrome is well documented, consisting in neurological symptoms and/or multivisceral disorder, the clinical expression of CDG Ia syndrome in adult patients is incompletely known.

Methods We report the features of 19 CDG Ia patients from 16 unrelated families, aged 12 to 39 years, detected by Western Blotting of serum transferrin, and confirmed by phosphomannomutase (PMM) activities and PMM2 gene mutations.

Results Fifty percent of the patients had neonatal symptoms. All the patients had variable developmental delay : all but two were able to walk, 5 were able to read and write some words, and one followed a normal school course. Nine had failure to thrive but only one had microcephaly. Epilepsy was observed in 6 patients, and stroke-like episodes in only 3. Neurological and ophthalmological signs were : (1) hypotonia in 16 patients, (2) ataxia in 14, (3) peripheral neuropathy in 5, (4) strabismus in 13, (5) ophthalmoplegia in 1, and (6) nystagmus in 1. Retinal changes were detected by electroretinogram in 4 of 6 cases. Extraneurological signs such as dysmorphism, subcutaneous lipodystrophy, thoracic deformity and thromboembolic episodes were not frequent, except late or absent puberty in females. Severe internal organ symptoms were absent. There were 12 different genotypes and no obvious genotype-phenotype correlation.

Conclusion This large series of teenagers and adults with CDG Ia syndrome highlights : (1) the great variability of disease expression, with the existence of mild forms, and (2) the non progressive course of CDG Ia syndrome after childhood.

C47. Congenital myopathy and brain migration defects with cutis laxa and a combined defect of glycan biosynthesis

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Congenital cutis laxa presents with decreased skin elasticity, connective tissue involvement and variable associated features. In most cases the genetic etiology is not known. Based on a diagnosis of CDG type IIx in a child with cutis laxa we performed a screening for disorders of protein glycosylation including a test for defective O-mucin type glycosylation in five patients with cutis laxa syndrome. Three patients from unrelated consanguineous marriages had an inborn error affecting the synthesis of both N-linked and O-linked glycans. These three children presented with a severe neonatal cutis laxa, skeletal and joint involvement, microcephaly, delayed closure of the fontanel, normal growth, severe hypotonia, developmental delay and neurological findings. All patients had an evident progress in the psychomotor development gradually. A significant improvement of the skin findings was observed with the development of fat-pads at an older age. Two of the three children were diagnosed with pachygyria and seizures and one with severe sensorineural deafness. In one patient with a brain migration defect, severe hypotonia and progressive congenital myopia a muscle biopsy was performed. This was combined with an immuno-staining for alpha-dystroglycan, a protein deficient in O-mannosylation defects, like Walker Warburg and muscle-eye-brain disease. Alpha-dystroglycan carries both O-mucin type- and O-mannoso-glycan groups. No muscular dystrophy, but a myopathy was confirmed in our patient and the immuno-staining with alpha-dystroglycan was normal, suggesting that the congenital myopathy and pachygyria occurs due to a developmental defect different from that in O-mannoso-glycan defects secondary to a dysfunction of a still unknown glycoprotein.

C48. Birt-Hogg-Dubé syndrome in Dutch families

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Background: Birt-Hogg-Dubé (BHD) syndrome is an autosomal dominant genodermatosis associated with skin fibrofolliculomas, pneumothorax, renal carcinoma and possibly other neoplasms. A *BHD* gene on chromosome 17p has recently been identified.

Study aim: To identify and characterize Dutch families with BHD syndrome, both at the clinical and molecular level.

Methods: Clinical evaluation of index patients with ≥ 10 skin fibrofolliculomas, collection of family data and screening for *BHD* germline mutations by DNA sequence analysis.

Results: We identified 28 subjects from 16 families who were clinically affected and/or carrier of a pathogenic germline *BHD* mutation. Pneumothorax was observed in three subjects, symptomatic renal carcinoma of mixed histological types was found in two patients at ages 39 and 40 years. Other malignancies were reported in five cases, no colorectal cancer was observed. *BHD* mutation analysis revealed pathogenic mutations in 10 out of 13 (77%) index patients tested. The frequently reported c.1740dupC mutation was found in three subjects. A novel nonsense mutation c.1065_1066delGCinsTA was detected in three index cases. One mutation carrier had minor skin features at age 28 years. One mutation carrier had no fibrofolliculomas or other BHD syndrome signs at age 67 years.

Conclusions: BHD syndrome is highly variable at the clinical level. *BHD* mutation carriers around the age of 30 years may have minor skin features. The yield of mutation analysis is high. The novel *BHD* mutation c.1065_1066delGCinsTA may be a Dutch founder mutation. Further insight into BHD syndrome will facilitate early diagnosis and direct risk counselling and preventive measures.

C49. Ethics and Genetics: An Islamic Perspective

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We are at a time of unprecedented increase in knowledge of rapidly changing technology. Such biotechnology especially when it involves human subjects raises complex ethical, legal, social and religious

issues. A WHO expert consultation concluded that "genetics advances will only be acceptable if their application is carried out ethically, with due regard to autonomy, justice, education and the beliefs and resources of each nation and community".

Public health authorities are increasingly concerned by the high rate of births with genetic disorders especially in developing countries where Muslims are a majority. Therefore it is imperative to scrutinize the available methods of prevention and management of genetic disorders.

Islam is a religion which encompasses the secular with the spiritual, the mundane with the celestial and hence forms the basis of the ethical, moral and even juridical attitudes and laws towards any problem or situation.

Islamic teachings carry a great deal of instructions for health promotion and disease prevention including hereditary and genetic disorders, therefore we will discuss how these teachings play an important role in the diagnostic, management and preventive measures including: genomic research; population genetic screening, including premarital screening, pre-implantation genetic diagnosis; assisted reproduction technology; stem cell therapy and genetic counselling.

C50. Towards cultural competence in cancer genetic counselling and genetics education: lessons learnt from Chinese-Australians

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In multicultural Australia, culturally determined attitudes to genetic testing and counselling may be incompatible with current genetics service provision. Australian guidelines for primary care medical practitioners on surveillance and referral to genetic counselling for breast, ovarian and bowel cancer are dependent on correct categorisation based on family history using Anglo-Celtic concepts (bilateral) of kinship. Many cultures have profoundly different understanding of kinship that is likely to impact on presentation of family history. Ethnographic studies to explore the diversity of beliefs about kinship, cancer and inheritance using Chinese-Australians, as a case. Participants in the first study attended two major familial cancer clinics in NSW¹. Fifteen Chinese-Australian community members were interviewed to confirm the findings.

English speaking competence does not necessarily correlate with holding "Western" biomedical views: those holding traditional beliefs most often had maintained strong links with the Chinese community. In addition, barriers to family communication can occur where there may be incompatibility between "western" and traditional beliefs about inheritance and kinship. Family history taking can also be impacted unless recognition is made of the patrilineal concept of kinship prevalent in this community.

The findings have been used to develop strategies for culturally competent genetic counselling for cancer susceptibility with Australian-Chinese patients and inform the development of genetics educational materials.

C51. Psychosocial impact of genetic counseling and testing for breast and ovarian cancer susceptibility genes.

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Last year our published data resulting from the study of psychosocial factors associated with genetic tests for predisposition to various types of cancer indicated the differences characteristic for patients in Czech Republic which might be explained by different social and cultural background. Further extension of this study allowed us to divide a group of female individuals tested for *BRCA1/2* mutation. The aim of presented study was to evaluate separately in this group overall satisfaction with genetic services, motivation for undergoing

genetic testing, information and support needs, screening and prophylactic surgery practices, test result disclosure and concerns about discrimination.

Questionnaires containing 20 multiple choice questions were distributed to female patients (N=77) who received pre- and post-test genetic counseling for *BRCA1/2* mutations testing. The questionnaires were returned back by 61 individuals and were divided into two groups: individuals affected by cancer (N=41) and asymptomatic individuals (N=20).

The preliminary results and high response rate (79%) show overall satisfaction with genetic services. Similarities in both groups were detected for further information needs (27%), professional psychological help needs (18%) and adherence to preventive screening practices (95%). Differences between cancer affected and asymptomatic individuals were observed in attitude towards prophylactic surgery (9% and 60% respectively), concerns about discrimination (14% and 30%) and principal motivation for undergoing testing. All individuals informed at least one member of family although the disclosure to distant relatives differs between affected (33%) and asymptomatic (20%) individuals. To support these findings statistic analysis also based on demographic data will be presented.

C52. Genotype-based screening for hereditary haemochromatosis in Germany

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We conducted a pilot study on DNA-based screening of hereditary haemochromatosis (HH) in Germany. 5882 insureds of the sickness fund Kaufmännische Krankenkasse - KKH requested information on this project. 3961 of these individuals provided blood samples. The analytic specificity of the tests methods with respect to the detection of homozygosity for the *HFE* mutation C282Y was 100% (95% CI: 99.95% - 100%), while the analytic sensitivity was 97% (95% CI: 92.5% - 99.2%). The direct costs ranged from 11.20 - 16.35 Euro per test method. 67 of the tested individuals were homozygous for C282Y. 42.6% of the homozygotes already knew their clinical diagnosis HH before sending the blood sample. Iron accumulation with clinical signs or symptoms of HH was present in 8 of 34 newly diagnosed C282Y homozygous individuals. 69.9% of the tested individuals believed that participation in the pilot study was probably beneficial for them and 1% thought that it was probably harmful. 94.6% judged their decision to have participated as right and 0.3% as probably wrong. 59.1% of the KKH members would generally accept predictive genetic testing and 3.7% were objected to such tests in principle. We conclude that the employed test methods for C282Y are robust, highly sensitive and specific. A DNA-based HH-screening program can be performed at reasonable laboratory costs and appears to be generally accepted and beneficial. Potential long-term negative psychosocial consequences should be taken into consideration when planning testing programs and can presumably be prevented by appropriate settings including genetic counselling and follow-up-services.

C53. Athlete's hearts or hypertrophic cardiomyopathies

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Distinct diagnostic criteria should prevent misdiagnoses of hypertrophic cardiomyopathy (HCM) in trained athletes with the so-called athlete heart; viceversa, clear criteria should guide diagnosis of HCM. These criteria include symmetrical versus asymmetrical, normal versus abnormal diastolic filling pattern, normal versus increased left atrial size, left ventricular wall thickness < or >16mm, left ventricular chamber normal or increased in athlete's heart and reduced in HCM.

We report six cases of current or past professional (n = 4), pre-competitive (n = 1) and amateur (n = 1) athletes who had long-lasting uncertain diagnostic work-up when they were involved in their sport activity. None of them complained of symptoms. The six cases share as common feature, the first suspect raised by the electrocardiogram (ECG). Then all underwent echocardiography, that was repeated up to 11 times in at least 5 different centres and by different operators, and finally to cardiac magnetic resonance imaging (MRI). MRI confirmed

the diagnosis of HCM. We identified pathological mutations in the Beta-MHC (MYH7) gene, troponin T (TNNT2) and myosin-binding protein C (MYBPC3), single in five (three MYH7, one TNNT2, one MYBPC3) and double in one (MYH7 plus MYBPC3). These cases document that when the diagnosis of HCM is uncertain, the identification of the pathological mutation of the disease gene closes that diagnostic work-up and contributes to definitely convincing patients and families on the diagnosis. The psychological impact of an uncertain diagnosis and the decision of stopping both competitive activity and training are hard, especially in "healthy" fully asymptomatic athletes.

C54. Genetics education and general practice: impact of an intervention

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We report here final data evaluating a practical genetics educational intervention developed for general practitioners (GPs) in Victoria, Australia, following an earlier needs assessment. The intervention includes a resource, available as a printed up-dateable folder, CD-ROM and online, and a highly interactive case-based workshop. Content and format of the resource, The Genetics File, was informed by GPs and includes sections on a number of topics, and focuses on practical aspects of GPs' roles. Resource/workshop content was developed in close collaboration with GPs, specialists and consumers. The educational intervention attracted maximum CPD points and 23 workshops were conducted, attended by 447 GPs. Evaluation included a validated questionnaire measuring categories of knowledge, attitudes, skills and behaviour, administered prior to workshop (n=332), then 1 (n=270) and 6 months (n=180) later. Referrals to clinical genetics services, genetic testing and support groups were also included in the evaluation. The mean rating of the intervention by GPs was 8.9 out of 10. Knowledge and behaviour were significantly improved (p<0.001) at 1 and 6 months, and attitudes, already positive at baseline, improved further (p=0.032 at 1 month). These combined measures assess the impact of the intervention on practice of genetic medicine by GPs. Recognition of its success has led to it forming the basis of a modified national Australian program, currently under development. Furthermore, there has been international interest in this resource as a model, and views on the intervention and strategies for implementing this were outlined in interviews with GP educators in the USA and UK.

C55. JARID1C, a novel gene involved in X-linked mental retardation, is frequently mutated

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More than 30% of mutations in families with non-syndromic X-linked mental retardation (NS-XLMR) seem to cluster on proximal Xp and in the pericentric region. In a systematic screen of brain-expressed genes from this region in 354 families with XLMR, we have identified 12 different mutations in JARID1C, including one frameshift and three nonsense mutations introducing premature stop codons as well as eight missense mutations changing evolutionarily conserved amino acids. Affected individuals present MR in the range from mild to severe, and short stature seems to be a prominent feature. In cell lines from two affected families, mRNA expression studies revealed an almost complete absence of the mutated JARID1C transcript, suggesting that the phenotype in these families results from a loss of JARID1C function. JARID1C belongs to the highly conserved ARID protein family. It contains several DNA binding motifs linking it to transcriptional

regulation and chromatin remodeling, a process, which is defective in various other forms of mental retardation. The mutation frequency of 3.4% in our patient cohort suggests that mutations in JARID1C are a relatively common cause of XLMR, and that this gene might play an important role in human brain function. Further studies will include the characterization of mice deficient in Jarid1c and expression profiling of the respective brain tissues. These investigations should shed more light on the pathogenesis of mental retardation.

C56. CDKL5 interacts with MeCP2 and it is responsible for the early seizure variant of Rett syndrome.

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MECP2 is responsible for both classic and preserved speech variant of Rett syndrome. We report here the identification of mutations in *CDKL5* gene, encoding a putative kinase, in 3 female patients: 2 early truncating mutations interrupting the catalytic domain (c.163_166del4; c.838_847del10) and 1 late truncating mutation (c.2635_2636del2). All 3 patients showed convulsions very early in life: in the first days of life in two of them and at 1,5 months in the third one. They all had stereotypic hand activities and the older patients, 9 and 8 years old respectively, showed acquired microcephaly. The clinical course of these patients is strikingly similar and they fulfill the criteria for the early seizure variant of Rett syndrome. We demonstrate that *CDKL5* is a nuclear factor whose expression in the brain of developing mice significantly overlaps the MeCP2 one. Moreover, we show *in vitro* and *in vivo* a direct interaction between MeCP2 and *CDKL5*. Functional characterization of *CDKL5* showed that *CDKL5* harbors an autophosphorylation activity, demonstrating that indeed it is a kinase. However so far we have been unable to demonstrate that *CDKL5* modifies MeCP2 under standard conditions. These results are in keeping with the fact that phosphorylation reduces MeCP2 dissociation from methylated DNA. If *CDKL5* were a MeCP2 kinase, it would be unlikely that *CDKL5* inactivation would lead to a phenotype similar to that produced by MeCP2 inactivation. In conclusion, our results indicate that a specific phenotype is associated with *CDKL5* and trace out a molecular link between MeCP2 and *CDKL5*.

C57. Use of murine models as a tool to dissect cognitive and physical phenotypes of Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS) is a developmental disorder occurring in ~1/20000 live births, caused by the heterozygous deletion of ~1.5Mb on chromosome 7q11.23. The commonly deleted region encompasses 26-28 genes flanked by segmental duplications that predispose to the mutational mechanism in humans. The only phenotype unambiguously associated with deletion of a gene is supravalvular aortic stenosis and the elastin gene. However, detailed deletion mapping on atypical patients with smaller deletion has identified two genes (*GTF2IRD1* and *GTF2I*), encoding members of a novel family of transcription factors, that are strong candidates for the main aspects of the cognitive phenotype.

To investigate the putative role of the general transcription factor gene *GTF2I*, we have used gene targeting in mice. In our model, the deletion of exon 2 of *Gtf2i* containing the start codon, abrogates to the translation of a modified TFII-I protein lacking the first 90 aminoacids. Previous *in vitro* studies have demonstrated that this mutated form is unable to bind DNA and does not activate transcription. Mutant mice are viable and display some craniofacial anomalies and mild postnatal growth retardation, obvious in the homozygous state. We are currently characterizing the neurocognitive and physical phenotype of these mutant mice, as well as the biochemical properties of the mutated

protein and its effect in the normal biology of the mouse embryonic fibroblast derived cell lines.

C58. Large genomic deletions influence expression levels of the non-hemizygous flanking genes

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An increasing number of human diseases, such as the Williams-Beuren (WBS) and the DiGeorge (DGS) syndromes, result from recurrent DNA rearrangements involving unstable genomic regions. Rearrangements are facilitated by the presence of region-specific low-copy repeats and result from nonallelic homologous recombination. It is assumed that these aneuploidies lead to underexpression of genes mapping to the commonly deleted regions. Furthermore, it is conceivable that these large chromatin rearrangements influence the transcription levels of genes that map centromerically or telomERICALLY to the critical region and the repeats, even if these genes are present in two copies. To test this latter hypothesis we used quantitative real-time PCR to accurately measure the expression of genes mapping to the 7q11.23 and the 22q11.2 regions. We studied in 20 WBS patients, 25 DGS patients and 10 controls the relative expression of 36 and 40 genes mapping within the WBS and the DGS critical region, their flanking repeats and neighboring regions in two different cell lines (skin fibroblasts and lymphoblastoids). As anticipated in WBS and DGS samples almost all of the genes mapping to the common deletion intervals show relative levels of expression decreased by 50%. Remarkably, a decrease in relative expression, albeit not as large, was observed for the non-hemizygous genes that map on both sides of the common deletion region. Our results suggest that in "genomic disorder" not only the aneuploid genes but also the genes that map close to the rearrangement should be considered as candidate genes for the specific features of these pathologies.

C59. Mutations in Rab3 GTPase activating protein (RAB3GAP)catalytic subunit cause Microphthalmia, Cataract, Microcephaly and Micropenis (Warburg Micro syndrome)

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Warburg Micro syndrome (MIM 600118) is a severe autosomal recessive disorder characterised by severe developmental abnormalities of the eye and

central nervous system and microgenitalia. To elucidate the pathogenesis of Micro syndrome we undertook a 10cM genome-wide linkage scan in eight consanguineous families. We mapped the *WARBM1* locus to 2q21.3 between markers D2S2282 and D2S2385 (with a maximum 2-point LOD score of 8.016 ($\theta=0$ at D2S1334) in five kindreds.

We then identified homozygous mutations in the catalytic subunit of RAB3GTPase activating protein (RAB3GAP) in 12 kindreds. In mouse we found significant expression of *RAB3GAP* in the developing eye and brain. RAB3GAP has a critical role in regulating the function of the four Rab3 family members that are implicated in neurotransmitter and hormone release by calcium mediated exocytosis. The Micro

syndrome phenotype demonstrates the that RAB3GAP for the normal development of the eye, brain and genitalia. We hypothesise that the underlying pathogenesis is a failure of exocytic release of ocular and neurodevelopmental trophic factors.

C60. Heterozygous mutations in the OTX2 gene cause structural malformations of the eye

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Major malformations of the human eye including microphthalmia, and anophthalmia are classical examples of phenotypes that recur in families yet often have no clear Mendelian inheritance pattern. Here we report heterozygous coding region changes in the homeobox gene OTX2 in eight families. The expression pattern of OTX2 in human embryos is consistent with the eye phenotypes observed in the patients, which range from bilateral anophthalmia to retinal dystrophy. Defects of the optic nerve, chiasm and brain were revealed by MRI in some cases. In two families the mutations appear to have occurred *de novo* in severely affected offspring and in two other families the mutations have been inherited from a gonosomal mosaic parent. These four families support a simple model in which OTX2 haploinsufficiency causes structural eye malformations. However a further four families display complex inheritance patterns suggesting that OTX2 mutations show reduced penetrance. The high incidence of mosaicism and reduced penetrance have implications for genetic counselling.

C61. 'Genetic PAP' at 5-12 gestational weeks: A new screening method for Down Syndrome?

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At present clinically available methods for prenatal chromosomal diagnosis (amniocentesis and CVS) are invasive, risky and costly. Screening techniques are cumbersome and expensive, and carry a false negative rate of 20%. We assessed the feasibility of utilizing exocervically retrieved extra villous trophoblast cells for screening for chromosome 21-aneuploidy and gender determination.

Methods- Samples of fetal cells were obtained from the external cervical os of pregnant women using a cytobrush. Eight slides were prepared using a cytopsin centrifuge. Immuno-histochemistry was performed using HLA-G and other anti- trophoblast antibodies. The marked cells were identified and scored. FISH for chromosomes X and Y was applied and the previously marked cells were returned to for diagnosis. FISH for chromosome 21 was applied in a second run to the same cells.

Results- Initially, samples were taken from 290 women prior to elective pregnancy termination. In 252 (87%) were trophoblast cells detected. Gender diagnosis concurred with that of the placental in 236 (93.6%). Subsequently 190 on-going pregnancies were screened. Trophoblast cells were detected in 164 (86.3%). Sex was correctly determined in 146 (89%). There were 2 cases of trisomy 21 and both were identified. There were 2 cases of missed abortions both initially diagnosed to be XY. Placental tissue karyotyping revealed both of them to be mosaics, 46,XY/45,XO and 47,XXY/46,XY .

Conclusion- this simple and inexpensive procedure may have the potential to replace currently available non-invasive screening techniques for Down syndrome.

C62. Molecular screening of Smith-Lemli-Opitz syndrome in pregnant women from the Czech Republic - present results

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive metabolic disorder. Clinical severity ranges from mild dysmorphism to severe congenital malformation and intrauterine lethality. SLOS is caused by the mutations in the gene for 3 β -hydroxysterol- Δ^7 -reductase (DHCR7), which maps to chromosome 11q12-13. The DHCR7 catalyzes the final step in cholesterol biosynthesis. It results in an abnormally low cholesterol level and increased level of 7-dehydrocholesterol (7-DHC). The incidence of SLOS is estimated around 1 : 10 000 to 1 : 40 000 in the Czech population.

Pregnant women undergo biochemical screening examination for Down's syndrome in the second trimester. When the level of human chorionic gonadotropin (hCG) and unconjugated estriol (uE3) appears low, the suspicion for SLOS is registered. A group of 143 fetuses with high risk for SLOS were examined by DNA analysis. A rapid PCR/RFLP technique and sequencing was used to detect mutations in DHCR7 gene. Cultivated amniocytes and/or blood of both parents were usually used as material for DNA isolation.

From the group of 143 pregnant women with the positive biochemical screening results, we found 3 fetuses with SLOS and 4 fetuses, which were heterozygotes for SLOS. Severe mutations IVS8-1G>C and W151X were identified in most of the cases. Moreover, in one case we found another mutation, W182C. Our results indicate that consequential molecular screening for Smith-Lemli-Opitz syndrome is important in pregnant women with low level of hCG and uE3.

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C63. Congenital diaphragmatic hernia and chromosome 15q26: Determination of a candidate region by use of array-based comparative genomic hybridisation and fluorescent in situ hybridisation.

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In the etiology of congenital diaphragmatic hernia (CDH), a severe birth defect, multifactorial inheritance, teratogenic agents, and genetic abnormalities all have been suggested as possible contributors. To define candidate regions for CDH, we analysed data collected on CDH cases since 1988. Karyotypic analysis of 200 patients showed numerical chromosomal anomalies in 7% and structural anomalies in 5% of the cases. The most frequent structural anomaly is a deletion of chromosome 15q, found in 3 patients. For this study, we have used these patients along with genetic material from four other, previously published, CDH patients with a chromosome 15q deletion. We used array-based comparative genomic hybridisation (array-CGH) and fluorescent in situ hybridisation (FISH) assays to determine the boundaries of the deletions in the patients. Most of the deletions extended towards the distal 15q terminus. However, by including data from two non-CDH patients with terminal 15q deletions, we were able to exclude a substantial portion of the telomeric region of chromosome 15. Moreover, one CDH patient harboured a small interstitial deletion. Together, this allowed us to define a minimal deletion region of approximately 5 Mb at chromosome region 15q26.2. Our data identifying this CDH-region on chromosome 15q26 will be presented.

C64. Antenatal presentation of Bardet-Biedl : the so-called Meckel-like syndrome

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Bardet-Biedl syndrome (BBS, OMIM 209900) is a multisystemic disorder characterized by progressive retinal dystrophy, postaxial polydactyly, obesity, hypogonadism, learning difficulty and renal dysfunction.

Other manifestations include diabetes mellitus, neurological signs, heart disease, and hepatic fibrosis. The condition is genetically heterogeneous and 8 genes have been identified during the 4 past years (*BBS1-BBS8*). In addition, a complex triallelic inheritance has been established in this disorder, i.e. in some families, three mutations at two *BBS* loci are necessary for the expression of the disease.

The only clinical features that can be observed prenatally include polydactyly, a kidney anomaly and hepatic fibrosis. Cystic kidney dysplasia, polydactyly, occipital encephalocele and liver anomalies (hepatic fibrosis and bile duct proliferation) also characterise Meckel syndrome (MKS). Based on these observations, we have decided to sequence all identified *BBS* genes in a series of 13 antenatal cases mostly referred as Meckel or Meckel-like (because of association of a kidney anomaly, polydactyly and/or hepatic fibrosis to a brain anomaly, or the familial history or the kidney histology). In 6 cases, we identified a recessive mutation in a *BBS* gene (respectively *BBS2*: 3 cases, *BBS4*: 2 cases and *BBS6*: 1 case). We also found heterozygous *BBS6* mutations in 3 additional cases. No *BBS1*, *BBS3*, *BBS5*, *BBS7*, or *BBS8* mutation could be identified in our series. These results extend the clinical spectrum of BBS with possible brain anomalies or severe cystic kidney dysplasia and strongly suggest that MKS and BBS are overlapping conditions.

C65. Vitamin K dependent Chondrodysplasia punctata

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Chondrodysplasia punctata (CDP) is a well known syndrome characterised by distinctive radiological features such as stippling in the epiphyses of the long bones and spine. The craniofacial abnormalities include a short nose with a flat nasal bridge, short columella and maxillary hypoplasia. There are various forms of CDP reported in the literature, e.g. Binder anomaly described as maxillonasal dysplasia, which is reported to be associated with prenatal phenytoin induced Vitamin K deficiency. We report 4 cases with exposure to various agents which reduce Vitamin K levels in the feto-maternal environment and result in CDP.

Case	Age	Sex	Exposure	Clinical Features
1	21 weeks fetal death	Male	Maternal anti-phospholipid syndrome	Poorly formed nose with maxillary hypoplasia.
2	2 years	Male	Phenytoin	Very small nose at birth with short, stubby fingers.
3	10 years	Male	Carbamazepine	Mild learning difficulties, a small nose and flat malar appearance
4	2 weeks	Female	Warfarin	Flat face and underdeveloped nose.

It is known that factors such as exposure to antiepileptic medication, anticoagulants, and maternal disease such as Systemic Lupus Erythematosus or Antiphospholipid antibody syndrome may cause CDP by affecting Vitamin K dependent processes. Matrix Gla Protein, a Vitamin K dependent protein is upregulated by Vitamin D in bone cells. A rat model shows that nasal septal growth retardation occurs because the warfarin induced extrahepatic vitamin K deficiency prevents the normal formation of the vitamin K-dependent matrix gla protein in the embryo.

Vitamin K dependent processes play a role in CDP. A detailed maternal history is important when diagnosing children with features of CDP. A skeletal survey is recommended. Treatment with Vitamin K in at risk mothers may reduce the risk of CDP.

C66. *CHD7* mutation in foetuses with CHARGE syndrome and expression during human development

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The acronym CHARGE refers to the association of congenital malformations first described by Hall et al. (1979) and includes ocular Coloboma, Heart malformation, choanal Atresia, Retarded growth, Genital hypoplasia, Ear abnormalities and/or deafness. The *CHD7* gene was recently shown to be mutated in 60 % of CHARGE postnatal patients (Visser et al. 2004). *CHD7* belongs to a large family of proteins thought to play a role in chromatin organization through their conserved "chromodomain".

We studied the coding sequence of the *CHD7* gene, in a cohort of 11 severely affected CHARGE foetuses. We identified a truncating mutation in 10 cases confirming the diagnosis. Anatomopathological examination and X-rays showed that semicircular canal hypoplasia as well as arhinencephaly are highly predictive diagnostic criteria. In addition to the C-H-A-E components of the acronym, facial dysmorphism or renal digestive and skeletal anomalies, should be considered to be minor diagnostic criteria for foetal CHARGE syndrome. In addition, we analysed the expression pattern of the *CHD7* gene during early human development. *CHD7* is widely expressed in undifferentiated neuroepithelium and mesenchyme of neural crest origin, and continues to be expressed toward the end of the first trimester in dorsal root ganglia, cranial nerves and ganglia, auditory and nasal tissues and the neural retina. Absent from the myocardium, bones and genital ridge, *CHD7* expression nevertheless correlates with defects in these areas because of its presence in neural crest cells and the pituitary.

C67. Functional significance of minor *MLH1* germline alterations found in colon cancer patients

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Hereditary nonpolyposis colorectal cancer (HNPCC) is associated with deficiency of the mismatch repair (MMR) machinery. One half of 450 mutations reported in an HNPCC database affect the *MLH1* gene. A significant number of those are of missense type, whose pathogenicity is difficult to interpret, and which are associated with a variety of clinical phenotypes. Here, the pathogenicity of 31 *MLH1* germline alterations, which were nontruncating and found in putative HNPCC families, was evaluated. For this, the expression level and subcellular localization of the *MLH1* variants, and the functionality of the mutated MutLa (*MLH1* and *PMS2*) complexes were studied. Furthermore, by correlating the genetic and biochemical data with clinical data, we tried to find some genotype-phenotype correlations. Among the 31 *MLH1* mutations, 19 affected either the expression or stability of the encoded *MLH1* protein. These variants also affected the subcellular localization of MutLa. Fifteen mutations disrupted the MMR function of *MLH1*. Comparative sequence analysis correctly predicted functional studies for 82% of missense variants. The aminoterminal *MLH1* mutations caused protein instability and defective MMR, whereas pathogenicity of the carboxyterminal *MLH1* mutations was caused by protein instability or defective assembly of MutLa. The genotype-phenotype correlations indicate that the mutations which affect both the function and stability of *MLH1*, are associated with typical HNPCC phenotypes. Instead, the *MLH1* mutations, which affect only mildly or cause no effect at all on the encoded protein, are found in families with variable disease phenotypes.

C68. Gene expression profiling of leukemic cell lines and primary leukemias reveals conserved molecular signatures among subtypes with specific genetic aberrations: identification of fusion gene-specific transcriptional profiles and expression pattern of tyrosine kinase-encoding genes

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Hematologic malignancies are characterized by balanced chromosomal abnormalities - translocations and inversions - that lead to deregulated expression of genes located in the proximity of the breakpoints or result in tumour-specific fusion genes. Although hematologic malignancies have been extensively studied, our understanding of how individual fusion genes elicit their leukemogenic properties is still quite limited. Today, the perhaps most widely used model system for studying the biological consequences of leukemia-associated chromosomal rearrangements is based on immortalized hematopoietic cell lines. Although cell lines are known to differ from both neoplastic and normal tissues, they provide powerful tools for investigating basic and applied aspects of leukemia cell biology. In the present study, we investigate a large number of cell lines, derived from all hematopoietic lineages, and analyze a large number of specific genetic aberrations. Using unsupervised algorithms, we show that immortalized hematopoietic cell lines maintain a gene expression signature correlating with their clinical subtype and primary genetic change. Moreover, by combining data from cell lines and primary acute leukemia samples, we show that the identified expression signatures in cell lines are present also in leukemic cells with identical genetic changes. By applying discriminatory analysis on the data set we have identified genes correlating with clinical subtype and genetic rearrangement. Finally, using supervised methods, the expression signature of genes encoding protein-tyrosine kinases was studied, identifying members that could be targeted using tyrosine kinase inhibitory drugs.

C69. Large scale variation copy number changes revealed by array CGH in CML

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Chronic myelogenous leukaemia (CML-BC) typically evolves in three distinct clinical stages: chronic and accelerated phases and blast crisis. The mechanisms responsible for transition of CML chronic phase into blast crisis remain poorly understood. While the acquisition of extra copies of chromosomes 8,19, the Ph and iso17q are recurrently found in the majority of CML-BC patients, the knowledge about aberrations at molecular level are sparse.

Here we present a genome wide screening by array CGH at a resolution of 1Mbp (Spectral Genomics Inc) for genetic imbalances of total 36 samples of CML and 10 CML cell lines. Using the same array CGH platform Ianfrate et al., (Nature Genetics, 2004) identified 255 loci that contained large scale variations (LCVs) among unrelated individuals, of which 24 were present in more than 10% of the cases. Our findings seen in > 20% of the samples fall into four main categories:

(i) LCVs affecting the loci reported in normal individuals (Ianfrate et al., 2004) but seen in the CML patients at much higher frequency (from 38% to 56%) and in combinations (on average 3.1 LCVs per patient);
 (ii) Unique to CML patients LSVs affecting four loci in 23 - 42% of the cases;
 (iii) Segmental loss of the 8p13 and amplifications at 8q24.12 regions;
 (iv) Novel features of the der (9) chromosome deletion map.

These data not only support the notion that the newly established heterogeneity underlines susceptibility to disease, but also provides new insight into the genetic aberrations associated with disease progression in CML.

C70. Chromosome radiosensitivity and apoptosis in breast cancer susceptibility

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BRCA1, BRCA2 and CHEK2 proteins 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 BRCA1, BRCA2 and CHEK2 mutations have an altered cellular phenotypic response to irradiation. We have investigated apoptosis, chromosome breakage and cell cycle dynamics in response to irradiation in peripheral blood lymphocytes in the following:- 53 BRCA 1/2 mutation carriers and age and sex-matched unaffected controls and three CHEK2 mutation carriers treated for breast cancer. The CHEK2 mutation carriers had a significant increased number of breaks per cell compared with the BRCA mutation carriers ($p<0.001$), age matched controls ($p<0.001$) and newly diagnosed breast cancer patients ($p<0.002$) using the classical G2 assay and also in metaphase spreads enriched for cells in S phase at the point of irradiation. The BRCA1 and BRCA2 mutation carriers also have an increase in radiation induced chromosome breaks and gaps compared with controls ($p<0.001$) using the S phase assay.

We have also investigated the apoptotic response to 4Gy in peripheral blood lymphocytes. Overall, there is no evidence for a reduction in radiation-induced apoptosis in BRCA1, BRCA2 and CHEK2 mutation carriers vs controls (mean apoptotic response 50% vs 53% vs 52% $P=0.15$).

There is no evidence of a difference in peripheral blood lymphocyte cell cycle dynamics between BRCA1, BRCA2 and CHEK2 mutation carriers and age-matched controls in response to irradiation. The results will be discussed in relation to possible mechanisms for cancer susceptibility.

C71. MicroRNAs as potential diagnostic and prognostic markers of disease

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MicroRNAs (miRNAs) are small, siRNA-like molecules encoded in the genomes of plants and animals that regulate the expression of genes by binding and modulating the translation of specific mRNAs. Several published reports have shown that the expression levels of some miRNAs are reduced in chronic lymphocytic leukemia, colonic adenocarcinoma, and Burkitt's lymphoma samples providing possible links between miRNAs and cancer. We developed methods for isolating and quantifying all of the known miRNAs in tissue samples. We have used these procedures to analyze tumor and normal adjacent tissues from patients with lung, colon, breast, prostate, bladder, thyroid, and pancreas cancer. Each tumor type can be readily distinguished from the accompanying normal samples based on the expression levels of 3-10 miRNAs. While each different tumor type was characterized by its own unique miRNA profile, it is interesting to note that several miRNAs appear to be up- or down-regulated in almost all tumor samples relative to normal adjacent tissue. This suggests that specific miRNAs might play roles in tumor suppression. Putative mRNA targets for a number of these miRNAs are known oncogenes providing a potential link with oncogenesis. We have developed reagents for up- and down-regulating specific miRNAs that have allowed us to identify genes (like RAS and MYC) and cellular processes (like proliferation and apoptosis) that are regulated by miRNAs. Further analysis of miRNA expression profiles in various disease states also suggests that these bioregulators play a role in disease progression.

C72. Cloning and Characterization of the Novel Tumor Suppressor Gene DEAR1

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The development of biomarkers for human tumorigenesis remains one of the most important goals of current cancer research. An exciting candidate has been identified by our laboratory that maps into a region highly implicated by LOH and cytogenetic studies in the development of numerous tumors, including breast, colon, prostate and lung. This novel gene, *DEAR1*, is a member of the TRIM family of proteins, known for their involvement in cellular differentiation, proliferation, development and apoptosis. Expression of *DEAR1* at high levels is limited to the ductal epithelium of numerous tissues; however, dramatic downregulation of *DEAR1* expression has been documented by immunohistochemistry in tumors of the breast (40% of samples), pancreas (62% of samples) and colon. Downregulation of *DEAR1* has also been observed by Northern and RNase protection assays in breast, renal, colon and lymphoma cell lines. Mutation analysis of breast cancer was undertaken in both cell line and tumor samples. Significantly, mutations were observed in 14% of samples examined (6/43). Complementation of a missense mutation in the breast 21MT cell line reverted growth in SCID mice from an aggressive, poorly differentiated tumor to a morphology reminiscent of ductal carcinoma *in situ*, suggestive that *DEAR1* participates in the early differentiation of ductal epithelium, the aberrant regulation of which could be critical to breast tumorigenesis. Thus, based on its ubiquitous expression in normal tissues, downregulation or loss of expression, specific mutation in breast cancer cell lines and tumors, and *in vivo* restoration of differentiation, *DEAR1* is a strong candidate tumor suppressor gene.

C73. The first genome wide linkage disequilibrium map

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Genetic maps describe patterns of recombination and can be used to identify genes affecting a particular phenotype. Recombination is measured in Morgans over a single generation in a linkage map, but may cover thousands of generations in a high resolution linkage disequilibrium map measured in LD units (LDU) (Maniatis et al, 2002). The HapMap project (IHMC, 2003) has led to a huge increase in the genotyping of single nucleotide polymorphisms (SNPs) that are the main source of LD information. We have used a subset of the September 2004 Caucasian HapMap data, consisting of 493,408 SNPs, to create the first genome-wide LD map which covers 99.7% (2,934 Mb) of the euchromatin. Recombination (Kong et al, 2004) accounts for 96.8% of the variance in LD in chromosomal arms and 92.4% in their deciles. The Malecot model predicts that the ratio of corresponding distances in LDU and Morgans estimates the effective bottleneck time *t* in generations (Zhang et al, 2004) which is constant between chromosomes. The LD maps estimate *t* as 1435 generations implying a bottleneck time of ~35,875 years, assuming 25 years per generation. Presumably, this low estimate reflects the partly cumulative effect of bottlenecks since the out-of-Africa migration. There are significant deviations in *t* between chromosome arms and deciles due to physical size, marker density and holes in the LD map. These differences may reflect inflated Morgan length due to underestimates of interference and the effects of selection, particularly on the X chromosome, and stochastic variation shortening LDU maps.

C74. Contribution of gene conversions to the genetic diversity of DNA segments

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Studies of human DNA diversity lead us to better understanding of human origins, demographic history and population structure; critical elements in genetic epidemiological quest of complex diseases. In 22 genes, promoter regions, arbitrarily defined as 2kb segments directly

upstream of the first exon, were screened by dHPLC in 40 individuals of African, Middle-Eastern, European, East-Asiatic and Amerindian descent. Polymorphisms were characterized by sequencing and genotyped in an extended panel of 80 individuals representing the same population groups. We found between 3 and 18 (average of 10) segregating sites in these 2kb segments. Nucleotide diversity estimates of 0.03 to 0.19 %, from both allele frequencies (average 0.08%) and the number of segregating sites (0.1%) fit well the genomic average. Haplotype worldwide diversity was 0.65 (0.27 to 0.84) and their numbers from 4 to 21 (average 10) correlated well with the count of segregating sites, as if recombination was negligible. Yet, in more than one third of these segments four-gametes test indicated the presence of recombinant haplotypes suggesting that some of these segments recombined more often than expected. Indeed, our estimates of the population recombination rate *Rho* exceed significantly the expected genomic average of about 0.4 per kb. Further comparative analysis of the *Rho* estimates in 2kb segments and along the whole sequence of 100 genes (from the University of Washington) suggests the important effect of gene conversion in the shaping of haplotype diversity. These haplotype characteristics may be of great importance in genetic linkage and association studies. *Supported by Genome Quebec/Canada.*

C75. LD mapping in diagnostic marker and gene discovery in acute myocardial infarction, hypertension and type 2 diabetes in founder population

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Traditional genome wide scans (GWS) using microsatellite markers with linkage analyses have not succeeded in finding many genes causing common polygenic diseases. With advancements in genotyping technology, the first dense enough marker maps have become available for LD mapping the entire human genome in genetically homogenous founder populations. We studied molecular etiology of acute myocardial infarction (AMI), hypertension (HT) and type 2 diabetes (T2D) in the East Finland population, one of the genetically and culturally most homogenous founder populations. We used the Affymetrix early access 100k SNP typing assay in sets of rigidly defined familial cases and extremely healthy controls, which both were drawn from the same extensively examined prospective cohort. We discovered associations of 1875 SNP markers and 902 genes with AMI, 1154 SNP markers and 436 genes with HT and 1962 SNP markers and 838 genes with T2D in either single-SNP or haplotype pattern mining analysis, with false positive probability of 5-20%. Multivariate diagnostic models including 8-24 SNPs and a few phenotypic measurements predicted about 95% of each disease. These preliminary findings include large numbers of novel disease-associated genes including potential drug targets and both non-coding regulatory and coding markers as well as several new etiologic pathways. We are confirming our findings by re-testing them in over 1000 cases and 1000 controls in each disease from East Finland and other more heterogeneous populations and by disease pathway analysis. We are happy to collaborate with research groups that are interested in testing our findings in their study populations.

C76. Plasma lipids: heritabilities, apoE, and effects of inbreeding in a genetically isolated population

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Despite progress in elucidating genetic determinants of dyslipidemia, most findings are based on families with extreme phenotypes. To further dissect these complex traits, an extended pedigree not derived on the basis of phenotype was ascertained from the population of a recent genetic isolate in the Netherlands. Thus far, 938 individuals, related within this single pedigree containing more than 60,000 people, have taken part in physical examinations and medical interviews in this ongoing study (the Erasmus Rucphen Family Study, ERF). Laboratory analysis of these subjects included determination of fasting plasma lipids. Heritabilities for total plasma cholesterol (TC), HDL, LDL, and TG, estimated using SOLAR and adjusted for multiple covariates, were found to be 0.35, 0.51, 0.29, and 0.21, respectively. Inclusion of

apoE genotype in the model decreased heritability estimates slightly yet significantly. A further analysis, which used regression to estimate plasma lipid means by inbreeding quartile, showed that TC and LDL levels increased when the extent of inbreeding increased. These trends were statistically significant ($p_{trend} = 0.006$ for TC and $p_{trend} = 0.025$ for LDL). In conclusion, our studies in an unselected pedigree show that genetic factors play an important role in the variation of plasma lipid levels. Common polymorphisms such as apoE account for only a small portion of this variance, but suggest that this population would allow for the localization of lipid genes with comparatively small effects.

C77. European distribution of cystic fibrosis (CFTR) gene mutations: correlation with disease incidence and application to screening

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Although there have been many reports on population specific distribution of *CFTR* gene mutations in the classical form of cystic fibrosis (CF), little attention has been given to integrating these findings into a global understanding. Thus, a long-term collaborative effort was launched to determine the distribution of *CFTR* mutations in the European populations in order to provide guidelines for routine screening, by the provision of detailed regional data. Final analyses were based on genotypes from 15606 CF patients (31212 CF chromosomes), using data compiled from over 102 original papers and from 82 collaborating European centres. The entire *CFTR* gene coding region was analysed in 73% of cohorts and altogether 729 mutations were detected. We also examined CF incidence, including regional mutational heterogeneity in a subset of populations via a cascade "data filtering" strategy. 422 alleles occurred only once, while in the remainder the 20 most common CF alleles comprise: F508del (63.45%); G542X (2.73); N1303K (2.22); W1282X (1.60); G551D (1.25); 1717-1 G->A (0.94); R553X (0.79); 621+1 G->T (0.59); 3849+10kb C->T (0.57); CFTRdel2,3/21kb/ (0.55); 2183AA->G (0.48); 394delTT (0.45); R1162X, 2789+5 G->A (0.44 each); 3659delC, R117H (0.43); I507del (0.36) and R347P, R334W (0.32). There are marked differences in regional / ethnic distribution of particular mutations among various populations. Moreover, statistics revealed a significant positive correlation between F508del frequency and the incidence of CF. From comprehensive data assessment we offer recommendations for optimized mutation screening strategies.

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C78. Phylogeographic analysis of mtDNA and Y chromosome lineages in Caucasus populations

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The Greater Caucasus marks a traditional boundary between Europe and Asia. Linguistically, it is one of the most diverse areas of the continental Eurasia, while genetics of the people living there is poorly understood. Mitochondrial DNA and NRY variability was studied in 23 Caucasus populations speaking Caucasus, Turkic, and Indo-European languages. Total sample comprised more than 1700 individuals on Y chromosome and more than 2100 individuals on mtDNA. Genetic outliers among the studied populations are relatively recently arrived Turkic speaking Nogays. The indigenous Caucasus populations possess generally less than 5% of eastern Eurasian mtDNA and Y-chromosomal haplotypes - in a profound contrast to the Turkic-speaking people at the other side of the Caspian, but not so dissimilar compared to the Volga-Turkic Tatars and Chuvashis or to the Anatolian Turks. Haplogroup frequency variation within the Caucasus populations, in some instances significant, appears to be caused primarily by specific aspects of the demographic history of populations. Phylogeographically, a particularly intriguing finding is the presence, though at low frequencies, of a predominantly northeastern African haplogroup M1 in many North Caucasus populations, though they lack sub-Saharan L lineages, relatively frequent in the Arab-speaking

Levant. Results obtained help to place the Caucasus populations into the scenario of the peopling of Eurasia with anatomically modern humans. Possible migration routes, peopling of steppe and mountain parts of the Caucasus and causes of high linguistic diversity presence in this region is analyzed in this study.

POSTERS

P0001. Peculiar symptoms of respiratory oxalosis without progressive course as a genetic complex disease

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Authors have reported on a new form of COPD as a genetic complex disease (G. Pospekhova, 1989). 80 probands (70 females, 10 males, sex ratio 7:1) and their relatives (56) were under our observation. The main diagnostic sings of this form were typical habitus, systemic oxalosis, hyperoxaluria and lack of progressive, progreidiens, inflammation course ((ROPC(-))). In 35 families we have observed ROPC(-) in 3 generations. Set of diagnostic laboratory features included intensive tolerance of peripheral blood mononuclears to the polyclonal antigen FGA in RSML test. Using RSML test monocyte migration was found to be stimulated vs. be inhibited in patients with ROPC(-) and their relatives independently of inflammation phase and age. Probands with ROPC(-) had low level of FGA sensitivity. Authors supposed immune cell alteration to be connected with family food preference providing underdoses of natural agents like FGA and be inherited.. Risk of ROPC(-) was found to correlate with genetic relationship: all first degree relatives have had RTML test inverted vs. 5% of the third degree relatives. Males with ROPC(-) have had some sings of hypogonadism and have been childless. Ascorbic acid has provoked disease manifestation or worsened its course. Our diagnostic set allows 1) to create group with high risk of ROPC(-) using RSML test, 2) make easier diagnostic process 3) put into practice predictive counseling, 4) start preventive correct medicinal management

P0002. Risk factors in abdominal wall defects

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The aim of this study was to describe the prevalence at birth of two abdominal wall defects (AWD) and to identify possible etiologic factors. The AWD came from 320,984 consecutive births registered for the period 1979 to 2002. Request information was obtained for cases and for controls. Hundred twenty one cases with AWD were analysed, 55.4 % were omphalocele and 44.6 % were gastroschisis. The mean prevalence rate for omphalocele was 2.18 per 10000 and for gastroschisis 1.76 per 10000. Associated malformations were found in 74.6 % of omphalocele compared with 53.7 % of gastroschisis; 28.3 % of fetuses with omphalocele had an abnormal karyotype, 44.7 % had a recognizable syndrome, association or an unspecified malformation pattern; 51.8 % of fetuses with gastroschisis had additional malformations that were not of chromosomal origin, but 1 case. Antenatal ultrasound examination was able to detect 45 (67.2 %) cases of omphaloceles and 31 (57.4 %) cases of gastroschisis. In 35 (52.2 %) cases of omphalocele and in 8 (14.8 %) cases of gastroschisis parents opted for termination of pregnancy. The overall survival rate was 16 (23.9 %) for omphalocele and 35 (64.8 %) for gastroschisis. Weight, length and head circumference at birth of infants with AWD were less than those of controls. The weight of placenta of infants with AWD was not different from the weight of placenta of controls. Gastroschisis was associated with significantly younger maternal age than omphalocele. Pregnancies with AWD were more often complicated by threatened abortion, oligohydramnios and polyhydramnios.

P0003. Life adaptation in 8 women with Rokitansky Séquence**S. Turyk;***Hospital Británico de Buenos Aires, Buenos Aires, Argentina.*

This report provides information about the adult life adaptation of 8 women with Rokitansky Sequence (R.S.), ranging now from 23 - 34 years of ages. R.S. is a defect of development of the caudal paramesonephric ductus, with incomplete or atretic vagina, rudimentary uterus, normal hormones and normal karyotypes.

The R.S. propositus were identified throughout the clinical examinations, hormonal and ecographic studies and chromosomal analysis of peripheral blood cells between 1982 and 1997. The diagnosis in the propositus was made between 11 and 18 years old. In order to obtain information about the life progress of participants, the following specific content areas were addressed: surgery made, educational progress, relationships, employment, sexual identity and orientation and leisure activity.

Results: In 5 of 8 propositus, the corrective surgery was made. In education, 4 patients made the secondary school (12 years degree), 2 patients made the University (16 years degree) and 2 patients did the master (18 years degree). 7 patients were employed full time and 5 propositus got married. All the patients present heterosexual identity. All propositus described a wide range of interests and choices. In conclusion, RS adults demonstrated good levels of cognitive and psychosocial competence and their adaptation has been positive.

P0004. Cytogenetic Evaluation of Infant Males with Mitral Valve Prolapse**S. Turyk¹, M. Sakurai¹, C. Croatto²;**¹*Hospital Británico de Buenos Aires, Buenos Aires, Argentina, ²Hospital Nacional Profesor Alejandro Posadas, Buenos Aires, Argentina.*

Mitral valve prolapse has been reported in patients with Klinefelter Syndrome. Because the clinical features in K.S. are not present until adolescence in boys with 47,XXY karyotype, we report here a detailed clinical history and karyotypes of 26 male infants with mitral valve prolapse. The ages of the studied children were 1-13 years, being the mean 5,8 years. 24 patients (92,4%) presented 46,XX karyotype and 2 patients (7,6%) presented 47,XXY karyotype. No phenotypic abnormalities (small testes or gynecomastia) , no developmental delays and no learning difficulties were observed in affected boys. Because of the significant medical and psychological benefits of early diagnosis of Klinefelter Syndrome, we recommend cytogenetic study for all cases of mitral valve prolapse among infant males, even without dysmorphic features

P0005. Structural central nervous system (CNS) anomalies in Kabuki make-up syndrome**T. Ben-Omran, A. S. Teebi;***Hospital for Sick Children, Toronto, ON, Canada.*

Kabuki make-up syndrome (KMS) [MIM147920] is a rare multiple congenital anomalies/mental retardation (MCA/MR) syndrome originally described by Niikawa et al. [1981] and Kuroki et al. [1981]. It is now well recognized worldwide with more than 350 cases reported in the literature. Most cases of KMS are sporadic, however several familial cases displayed autosomal dominant transmission. Duplication of chromosome 8p22-8p23.1 was recently demonstrated in multiple cases of KMS from different races, suggesting a possible common etiologic basis. KMS is characterized by a peculiar facial appearance, mild to moderate mental retardation, short stature, skeletal anomalies, and unusual dermatoglyphic patterns. The most striking feature is the peculiar face that consists of long palpebral fissures and long eyelashes, eversion of the lateral one-third of the lower eyelid, arched and notched eyebrows with sparseness of their lateral one-third, and prominent and antverted ears. Since the facial appearance often reflects the developing brain, it is not unexpected to observe a relatively higher frequency of CNS anomalies compared with disorders with minimal facial dysmorphism. A number of reports have indicated an apparent increased frequency of such anomalies. Here we report on three unrelated patients with typical KMS and structural CNS anomalies and, briefly review literature pertaining to these anomalies in KMS patients.

P0006. Clinical Manifestations associated with Familial Partial Trisomy 11**T. Ben-Omran¹, E. Kolomietz², I. E. Teshima³, M. Mah⁴, D. Chitayat⁵;**¹*Division of Clinical and Metabolic Genetics, the Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, ²Department of Laboratory Medicine and Pathology, Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada, ³Department of Pediatric Laboratory Medicine, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada, ⁴Department of Psychiatry, Toronto General Hospital, University of Toronto, Toronto, ON, Canada, ⁵Division of Clinical and Metabolic Genetics, Hospital for Sick Children, The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, ON, Canada.*

The etiology of mental retardation, often presenting in childhood, is unknown in approximately 50% of the cases. Cytogenetic analysis is regarded as the mainstay in the diagnostic process. Identifying a chromosomal abnormality in patients with mental retardation provide necessary information for genetic counseling regarding prognosis, recurrence risk, and prenatal diagnosis options. In addition, determination of the clinical manifestations associated with abnormal karyotypes is an important tool for the identification of genetic disorders and the genes causing them. Here we report on a family with three members having mild to moderate mental retardation with learning disabilities, microcephaly, subtle dysmorphic features and mood disorders. The finding of the same mood disorder in all affected members of this family is important in determining genes associated with these disorders which are thought to have multifactorial inheritance. Cytogenetic and genomic microarray analysis revealed an unbalanced karyotype with duplication of a segment derived from the long arm of chromosome 11. This duplication was examined by genomic microarray and confirmed by FISH. Within this duplicated region, estimated to be 12 Mb region of DNA, four brain-expressed genes appear to be particularly promising based upon their likely functional roles: i) NAALAD2, N-acetylated alpha-linked acidic dipeptidase 2; ii) GRM5, glutamate receptor, metabotropic 5; iii) MTNR1B, melatonin receptor 1B; iv) PANX1, pannexin 1. Possible role of these genes in the clinical manifestation in this family will be discussed.

P0007. Cerebrofaciothoracic syndrome**K. Boduroglu, Y. Alanay, E. Tuncbilek;***Hacettepe University, Ankara, Turkey.*

Cerebro-facio-thoracic dysplasia is a rare congenital anomaly/mental retardation syndrome described by Pascual - Castroviejo in 1975. Autosomal recessive inheritance has been suggested because of parental consanguinity in 2 of the three reported families. The features include facial dysmorphism, multiple malformations of the vertebrae and ribs, and mental retardation. Costovertebral abnormalities are similar to those of spondylocostal dysostosis, Robinow syndrome and cerebrocostomandibular syndrome. Only a few cases have been reported since first report.

We report on a 3 year old girl with cerebro-facio-thoracic dysplasia. She was born at term from a 22 year old healthy mother. Birth weight was 4,000 g, birth length 49 cm. She was the first child of first cousin parents. Cleft lip and palate was noticed on first physical examination after birth. Before her referral to our department at age 3, she had had corrective operations for cleft lip and cleft palate at 8 and 18 months, respectively. On physical examination at age 3, weight was 13,500 g (50th centile), height 95 cm (50-75th centile) and OFC 49 cm (25-50th centile). Brachycephaly, hypertelorism, epicanthic folds, low set ears, and short neck were noticed. Radiographs showed cervical and thoracic vertebrae anomalies and bifid and abnormal ribs.

This is another case that suggests autosomal recessive mode of inheritance for cerebro-facio-thoracic dysplasia.

P0008. Spinal Muscular Atrophy. Towards a mutational spectra in Western Sweden.**E. L. Arkblad¹, K. Berg¹, J. Wahlström¹, N. Darin², M. Tulinius², M. Nordling¹;**¹*Clinical Genetics, Sahlgrenska, Goteborg, Sweden, ²The Queen Silvia Children's Hospital, Sahlgrenska, Goteborg, Sweden.*

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder caused by degeneration of the anterior horn cells of the spinal cord. SMA is divided into type I, II and III on the basis of age at onset and the maximum motor function achieved. The incidence has been estimated to be 5×10^{-5} , for SMA types I and II, in western Sweden. The disorder is

caused by the homozygous deletion/inactivation of the Survival Motor Neuron Gene (SMN1). The severity is in part related to the number of pseudogenes (SMN2) present and may be related to the size of the deletion. A higher number of SMN2-genes correlate with a milder or even asymptomatic phenotype.

A new molecular genetic technique, Multiplex Ligation-dependent Probe Amplification (MLPA), makes it possible to simultaneously determine the gene-copy number for SMN1, SMN2 and the nearby genes BIRC1 and GTF2H2. This is also a method suitable for carrier detection. Using this method in the clinical setting gives us a spectrum of SMA mutations in Western Sweden. During 2003-2004 we have analysed 110 individuals of whom 30 were patients with SMA, 34 were carriers and 46 had at least 2 copies of SMN1 and were therefore considered normal (33 diagnostic, 13 carrier tests).

In a few patients, with strongly suspected SMA and only 1 copy of SMN1, further analyses of the SMN1-gene have been made. So far one point mutation and one partial deletion have been identified. Genotype / phenotype studies are being performed on this relatively well-characterised cohort of patients.

P0009. Molecular Study Of *PKD1* & *PKD2* Genes By Linkage Analysis In Several Iranian Families With Autosomal Dominant Polycystic Kidney

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Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder with genetic heterogeneity. Here we report the first molecular genetic study of ADPKD and the existence of locus heterogeneity for ADPKD in Iranian population by performing linkage analysis on 15 affected families. Eleven families showed linkage to *PKD1* and two families showed linkage to *PKD2*. In two families, *PKD1* markers are common in all affected members but *PKD2* markers were not informative. The results of this study demonstrate significant locus heterogeneity in autosomal dominant PKD in Iran. Analysis of clinical data confirms a milder ADPKD phenotype for *PKD2* families. Our results showed relatively high heterozygosity rates and PIC values for some markers, while the most informative markers were KG8 and 16AC2.5 for *PKD1* gene and AFM224x6 for *PKD2* gene.

P0010. Aarskog Syndrome

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We have two families with this syndrome. The first family have two affected son. The second one have an affected son and a daughter with mild form of syndrome.

Both families mothers have hypertelorism. The sons signs were hypertelorism, short stature, broad filtrum, round face, cleft lip and cleft palate, down slanted palpebral fissure, broad forehead, short hands and feet, simian crease, clinodactily, brachydactily, interdigital web, shawl scrotom, some degree of learning disabilities and mental retardation.

It is a rare Xlinked semi dominant syndrome. Most cases have been males and transmission can occur through mild affected females. Gen map by linkage analysis is Xq1.3 and Xp11.2.

These families came to our clinic to know about the recurrence risk and seeking advise.

P0011. Is there any association between *ESRB* gene polymorphism and male infertility in Iranian infertile men?

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It is clear that a significant proportion of infertile male with azoospermia and severe oligospermia have a genetic etiology for reproductive failure. Cytogenetic analysis and Y-chromosome micro deletion and other conventional methods such FISH had major effects on finding the causes of infertility in last decade, but still after performing all these experiments in some cases we do not have clear answer for our patients. Therefore it would be advisable to find new clues for this old but interesting problem.

In this study we performed an association study between *RsaI* and *AluI* single nucleotide polymorphism in the *ESRB* gene of infertile patient in comparison with normal fertile male control.

From 180 infertile male patients referred to our center after ruling out all the known causes of the infertility such as chromosomal abnormalities, Y-chromosome micro deletion and other pathologic disorders, 5 ml peripheral blood were obtained and DNA were extracted. PCR amplification of the polymorphic region was carried out and after running the PCR products on 2% agarose gel, the frequency of the polymorphism were calculated.

A 2.7% times higher frequency of the heterozygous *RsaI* genotype was found in men with oligospermia compared to controls ($p=0.004$). In the other hand, the proportion of homozygous *AluI* genotype was about 0.3% in comparison to our normal control ($p=0.04$). Our result could suggest that these SNP could play an important role in the spermatogenesis process in male.

P0012. Recurrence of anophthalmia within the same sibship: evidence for germline mosaicism for a heterozygous *SOX2* mutation

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The etiology of anophthalmia is complex with environmental, syndromic, genic and chromosomal origins reported. Heterozygous mutations within the *SOX2* gene has been identified in anophthalmia with associated extraocular features including neurological abnormalities (psychomotor delay, myopathy, spastic diplegia), external genitalia abnormalities and tracheo-esophageal fistula. Here we report the first recurrence of *SOX2*-associated eye malformation of the anophthalmia/microphthalmia spectrum within the same sibship from unaffected unrelated parents. Severe bilateral microphthalmia and unilateral cryptophthalmia was associated with cerebral structural abnormalities, including corpus callosum agenesis, enlarged ventricles, hypoplastic inferior cerebellar vermis and hypoplastic white matter in case 1. Bilateral anophthalmia was associated with psychomotor retardation but normal cerebral MRI in case 2. A heterozygous *SOX2* mutation (N46K), located at the 5'end of the HMG box and presumed to result in loss of function, was found in case 2. The mother carried the same mutation but as a mosaic. A segregation analysis revealed that some unaffected children carried the same maternal chromosome than our proband but did not carry the mutation. Those results explained both the eye malformation and the "pseudo-recessive" inheritance pattern in the family. This is the first case of gonadal mosaicism for *SOX2* and this has significant implications for genetic counseling in such eye malformation.

P0013. A New Mitochondrial Disease in Bulgaria

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Mitochondrial diseases affect 6-17/100 000 of many populations but the frequency of these disorders in our country is unknown yet. About 15 cases have been registered up to now. We report a case with a new mitochondrial disease. Diagnosis was confirmed by investigating mtDNA with the PCR-SBT method. The patient was a 4 year old boy, born after a normal pregnancy and delivery. On the 3rd day after the birth his general condition worsened. He had hypotonia, clonic convulsions, tachypnea, hepatomegaly, clonus, missing reflexes of Moro and Robinson. Biochemical investigation showed metabolic alkalosis, hypoglycemia, hyperammonemia, elevated transaminase and alkaline phosphatase. U/S of the brain showed oedema cerebri and hyperdensity. EEG showed paroxysmal activity. Despite a low protein diet and anticonvulsive therapy, he developed quadriplegia spastica and West syndrome. MRI at 1 year of age showed apical and occipital encephalomalacia. PCR-SBT revealed mtDNA mutations: A5315G, A89016G, G9300A. The last is in the gene for cytochrome C3 oxidase and causes an aminoacid change of alanine to threonine. All these mutations are novel. These data confirm a new mitochondrial disorders. The investigation are continuing.

P0014. Nevus sebaceus of Jadassohn - a case with severe structural brain anomalies; clinical and pathological correlations

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Nevus sebaceus of Jadassohn or linear/epidermal nevus syndrome is a rare condition that belongs to neurocutaneous hamartoses. Molecular base of disease is still unexplained. Here we report on a boy of gypsy origin with severe neurologic involvement and early death.

The boy was from the first pregnancy of a healthy young nonconsanguineous couple. The pregnancy was unremarkable until the 7th month, when the ventriculomegaly was found on ultrasound. Delivery was in the 38th week by C-section, the birth weight was 2940 g, length 49 cm, head circumference 38 cm. Clinical and imaging findings included right-sided facial hemihypertrophy, linear nevus sebaceus covering the right side of the face, right-sided dysplastic hemimegalencephaly with schisencephaly, lissencephaly and hydrocephalus, and atrophy of left hemisphere. Moderate clonic seizures started from the 3rd day of life and progressed to severe epilepsy. EEG record (epileptic discharges with burst suppression) together with clinical neurological findings showed evidence of early infantile epileptic encephalopathy. There were no other system malformations; ophthalmologic examination did not detect any abnormalities. Chromosomal analysis revealed normal karyotype. Family history was negative for brain malformations, mental retardation, epilepsy and skin anomalies.

In the age of 4 month the patient suddenly died at crib at home. Forensic autopsy determined severe brain oedema as the cause of death. Macrosections of brain confirmed the structural anomalies listed above. Unusual histological findings included distortion of laminar character of cortical cytoarchitectonics, primitive layers of cortical neurocytes, spongiiform changes of grey and white brain matter, and focal gliosis.

P0015. High resolution comparative genomic hybridisation analysis for detecting small constitutional chromosome abnormalities

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Comparative genomic hybridization (CGH) is a molecular cytogenetic technique that allows genome-wide analysis of DNA sequence copy number differences. Recently, modification of CGH with increased resolution down to 3-5 Mb has been reported. This high resolution CGH (HR-CGH) allows characterization of redundant and missing cytogenetic material often unrecognizable by G-banding, and enhances sensitivity and specificity in the detection of aberrations.

Although, the application of CGH is mainly in the field of cancer genetics, its use in clinical cytogenetics also increases. In clinical cytogenetics CGH has been particularly suited for simplification of identification and characterization of intrachromosomal duplication, deletions, unbalanced translocations and marker chromosomes.

In this work we are reporting about the application of the recently developed method of HR-CGH for clarification of small terminal imbalances in 4 patients with abnormal karyotypes and dysmorphic features. The characterization of additional material on 1p, 4p, 6p and 17p chromosomes was performed and der(1)(1;11), inv(4p), dup(6p) and der(17)(11;17) were confirmed by using HR-CGH. The results were subsequently confirmed by FISH and spectral karyotyping (SKY).

We conclude that HR-CGH can be used as an excellent diagnostic tool for clarification and identification of chromosomal imbalances not apparent from a routine cytogenetic analysis.

P0016. A child with Feingold syndrome (tracheo-oesophageal fistula, oesophagus and duodenal atresia, and dysmorphic features) with an interstitial deletion of 2(p24p25)

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Feingold syndrome is an autosomal dominant condition with microcephaly, limb abnormalities and oesophageal or duodenal atresia. The gene maps to 2p23-p24 but remains to be identified.

We report a girl delivered by Cesarian section after 34 weeks gestation due to asphyxia, with a birth weight of 1560g. She was operated on 2 days old for oesophagus atresia and tracheo-oesophageal fistula. An annular pancreas, duodenal atresia and Meckel's diverticulum were also found. Furthermore, a small muscular VSD and ASD were demonstrated. She had dysmorphic features with sandalgap and simian creases bilaterally. A conventional chromosome analysis in the neonatal period was normal. 12 months old she was below - 4 Standard Deviations for height, weight and head circumference. She had a dysmorphic facies with hypertelorism, epicanthus and fine arched eyebrows. There was clinodactyly of second and fifth fingers. Development was retarded.

High resolution Comparative Genomic Hybridisation (CGH) and subsequent FISH analysis demonstrated a del(2)(p24p25) *de novo*. In order to generate a more detailed map of the deleted area, array based CGH was performed using a genomic microarray representing the human genome at approximately 1Mb resolution. The deleted area was delimited to a region of approximately 10 Mb in 2p25-p24 and extended from RP11-542B5 to RP11-262D22.

P0017. A screening strategy based on the analysis of Beta-Myosin Heavy Chain, Cardiac Myosin Binding Protein C and cardiac Troponin T for Hypertrophic Cardiomyopathy.

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Background. Mutations causing hypertrophic cardiomyopathy (HCM) have been described in ten different genes of the sarcomere. However, three genes account for >50% of known mutations: Beta-Myosin Heavy Chain (*MYH7*), Cardiac Myosin Binding Protein C (*MYBPC3*), and Cardiac Troponin T (*TNNT2*). Thus, we prospectively assessed a screening strategy based on the comprehensive evaluation of *MYBPC3*, *MYH7* and *TNNT2* in a consecutive population with HCM. **Methods.** A total of 163 unrelated HCM patients were screened in *MYBPC3*, *MYH7* and *TNNT2* genes by *DHPLC* and automatic sequencing. **Results.** We identified 54 mutations in 82 index patients (50% of the study cohort); 37 were novel. The prevalence rates for *MYBPC3*, *MYH7* and *TNNT2* were 34%, 12% and 4%, respectively. *MYBPC3* mutations were 35, including 7 frameshift, 7 splice-site and 3 nonsense. All were "private" except E542Q, IVS24-2 A>G, insC1065, R502Q, M555T, and IVS12+1G>A, which were present in 2-5 unrelated patients. Moreover, E258K was found in 12% of the patients, suggesting a founder effect. One patient was homozygous for a *MYBPC3* mutation. *MYH7* mutations were 13, all missense; 8 were novel. In *TNNT2*, only 6 mutations were found. Ten patients carried a double mutation within the *MYBPC3* gene (n=6), or were double heterozygous for a *MYBPC3* mutation associated with a mutation in *MYH7* (n=3) or *TNNT2* (n=1).

Conclusions. Mutations of *MYH7*, *MYBPC3* and *TNNT2* accounted for 50% of patients in a consecutive HCM cohort. Thus, the combined analysis of these 3 genes represents a rational and cost-effective initial approach to genetic screening of HCM.

P0018. VMD2 gene mutational screening in Italian families: identification of a novel mutation associated with Best Maculopathy

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Background: Best's disease is an autosomal dominant, early-onset form of macular degeneration. *VMD2* gene, considered responsible

for the disease, codifies the protein Bestrophin: the function of this protein is not known. In the present study we screened for mutations the VMD2 gene in Italian patients with Best maculopathy.

Methods: Seven families with Best disease were recruited from central and southern Italy, and family members were evaluated by standard ophthalmologic examination. DNA samples were analyzed for mutations in VMD2 gene by DHPLC approach and direct sequencing techniques.

Results: Some mutations of the VMD2 gene have been detected in all the affected patients and in some unaffected relatives. Most of the mutations have already been described in the literature; a novel mutation (R218G) was detected. It is also interesting to remark that in this series the same mutation was associated with some differences in clinical phenotypes. In fact in one family the R218C mutation was associated with the onset of choroidal neovascularization (CNV) in the affected mother and her son, while no CNV was reported in another family sharing the same mutation. Another family with the R25W mutation showed a multifocal location of the vitelliform deposits, while another family with the same mutation showed a typical isolated vitelliform disc in the macular area.

Conclusions: Some mutations of VMD2 gene have been detected in some Best's disease families; among them there is a novel missense mutation. In spite of the small number of studied families it was possible to remark a certain phenotype heterogeneity.

P0019. Stargardt Disease in Italian patients: identification of nine novel mutations in ABCR gene

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Purpose: Stargardt disease (STGD) is a progressive juvenile-to-young adult-onset macular degeneration with severe reduction of central visual acuity and normal peripheral vision..Mutations in ABCR gene are responsible for autosomal recessive Stargardt disease (arSTGD). In this study we determined the mutation spectrum in ABCR gene in a group of Italian patients with arSTGD.

Methods: Thirty-two families from central Italy, some members of which were affected by arSTGD, were clinically examined. DNA samples were analyzed for mutations in all 50 exons of the ABCR gene by DHPLC approach and direct sequencing techniques.

Results: All the affected subjects showed bilateral central vision loss with macular atrophy and yellow-white flecks at the posterior pole, typical dark choroid in fluorescein angiography, normal electroretinogram, normal caliber of retinal vessels, no pigmented bone spicules in the retinal periphery. In all these patients we reported some mutations of ABCR gene. Some of these mutations have been already described and among them G1961E was the most frequent in our series. Nine novel mutations were identified: five missense mutations (N96K, T970P, F1015I, P1484S and L2221P); one nonsense mutations (Q21X); two small deletions (5109delG, 5903delG and 6750delA). These mutations were not detected in 150 unaffected control individuals (300 chromosomes) of Italian origin.

Conclusions: Some novel mutations in ABCR gene in STGD patients were reported. These data confirm the extensive allelic heterogeneity of the ABCR gene, in agreement with previous observations in patients with Stargardt disease from Italy.

P0020. Genetic testing in familial melanoma: uptake and psychological implications

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We have started offering genetic testing for p16-Leiden (a CDKN2A founder mutation) and so far we have found no adverse effects. Uncertainties regarding the risk estimates associated with melanoma gene defects have prompted the International Melanoma Genetics Consortium to consider genetic testing premature unless conducted within a strict protocol. Accordingly, we have started offering predictive genetic testing in the p16-Leiden families for which we have well-

defined risk characteristics. The gene defect is associated with increased risks of the treatable and preventable cancer melanoma and with the non-treatable and non-preventable pancreatic cancer. Given these disease characteristics, decision-making is thought to be even more difficult and therefore we aimed to evaluate uptake, motivation and psychological implications of genetic testing.

Of the 403 eligible subjects, 166 (41%) opted for counselling. Variables significantly predictive for counselling uptake were being a parent, higher prior risk and older age. A total of 127 (77%) counselees finally opted for genetic testing. Age was the only significant predictor for test acceptance. Of the counselees, 94 (57%) were included in the psychological study. Counselees reported lower distress levels after the first counselling session than those reported in other oncogenetic testing settings like HBOC and FAP, despite being informed about pancreatic cancer. In addition, these levels were not clinically elevated. Test acceptors had more positive expectations of the test than decliners.

We report a relatively high uptake rate for p16-Leiden testing and no clinically worrisome levels of distress after the first counselling session.

P0021. New mutations in the Protein Kinase Cy gene associated with Spinocerebellar Ataxia Type 14 (SCA14)

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Objective To evaluate the frequency for PKCy gene mutations and determination of the associated phenotype.

Background Autosomal dominant spinocerebellar ataxias (ADCA) are a heterogeneous group of movement disorders characterized by progressive ataxia that is variably associated with other neurological signs. In addition to the known expansions of repeated sequences, six point mutations have been described in the gene encoding protein kinase Cy (PKCy) accounting spinocerebellar ataxia (SCA) 14.

Methods Direct sequencing of exons 1, 4, 10 and 18 of the PKCy gene in 238 index cases from ADCA families that did not carried expansions in the SCA 1, 2, 3, 6, 12, 7, 17 and DRPLA genes.

Results Six missense mutations, including 5 newly reported, were identified in exon 4, 10 and 18. The mutations segregated completely with the disease and were located in highly conserved regions of the protein, except for a single mutation only conserved among mammals. The mutations were absent in 560 control chromosomes.

The phenotype of the affected individuals consisted in a slowly progressive pure cerebellar ataxia with few additional neurological features (mild cognitive impairment, myoclonus, sphincter disturbances and decreased vibration sense) in some patients. The mean age of onset of was 33.5 ± 14.2 years (range 15 to 60 years).

Conclusion Although SCA 14 remains a rare form (6/238) of ADCA in families without the classical repeat expansions, mutations in the PKCy gene should be considered in patients with slowly progressive ADCA associated with myoclonus and/or mild cognitive impairment.

P0022. Bilateral upper extremity deficiency, unilateral absence of fibula with oligodactyly and zygodactyly:a new clinical entity?

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We report a boy with multiple congenital extremity anomalies. The propositus is four years old. His parents, are both from the same region of Turkey but not relatives. He has reduction deformity of forearms and both hands. Especially, the distal part of the left leg was shorter than the right leg. He has oligodactyly on the left foot and zygodactyly on the right foot. Prominent radiologic findings include bilateral aplasia/hypoplasia of radii and ulna, absent carpals, nearly complete absence of metacarpals and phalanges. Both tibias were thickened and the left tibia was shorter than the right one. No fibula was observed on the left side. He has four metatarsals, four toes on the left foot, whereas, he has four metatarsals, five toes on the right foot. This combination of features has not been described previously, and may present a new clinical entity.

P0023. Outcome of a pregnancy of a couple with Noonan syndrome in both partners

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We report the outcome of the first pregnancy of an unrelated couple with a clinical diagnosis of Noonan syndrome and a missense PTPN11 mutation in both partners. In addition to the 50% risk of having a baby with Noonan syndrome and one PTPN mutation, the couple also have a 25% risk of compound heterozygosity which has never been reported in the literature before, and for which the phenotype is unknown. The pregnancy resulted in an early fetal demise at 12 weeks. Hydrops and cystic hygroma were observed on a dating scan, and a subsequent fetal medicine scan showed that the fetus was no longer viable. Cytogenetic and molecular studies confirmed compound heterozygosity. Our case suggests that homozygous Noonan syndrome is a severe condition presenting prenatally with severe hydrops and intrauterine death.

P0024. Phenotypic variability in families with mesiodens

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Mesiodens is the most common supernumerary tooth also known as an extra incisor located in the midline between the two permanent central incisors. Mesiodens usually occurs singly and can be isolated or associated with other anomalies. Often does not erupt. The literature reports several theories, including the genetic one, concerning the cause of mesiodens but subject is still debated. **Objective:** to identify the genetic cause of clinical variability of mesiodens as observed in our cases. **Subjects and Methods:** investigation of mesiodens was carried out on 17 Caucasian patients ranging in age from 8 to 23 years; based on the phenotypic presentation of mesiodens (shape, size, location and eruption) we classified the patients in four groups; the mesiodens was diagnosed by oral and radiographic examinations; family study, cytogenetic and molecular analyses were performed. **Results:** 15 patients presented mesiodens as an isolated trait; the affected members within the same family often exhibited variability in clinical presentation; 2 patients exhibited mesiodens in association with other dental anomalies; family history of the patients suggested either the mendelian pattern of inheritance (autosomal dominant with complete penetrance or X-linked dominant) or an unclear pattern of transmission; no cytogenetic aberration were found. **Conclusions:** mesiodens is an inherited developmental anomaly; intrafamilial variability could be explained by variable expressivity of a single mutant gene or by the effect of modifier gene; interfamilial variability could be well explained by locus heterogeneity rather than the molecular heterogeneity. Early diagnosis of familial mesiodens prevents the clinical complications.

P0025. Poikiloderma, Alopecia, Bilateral Optic Nerve Atrophy, Cerebral Calcification, and Bone Marrow Suppression: Severe Rothmund Thomson Syndrome?

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A Saudi Arab girl with poikilodermaous skin changes, generalized alopecia, bilateral optic nerve atrophy, cerebral calcification, and significant delay of growth and development is described. The girl developed bone marrow suppression requiring frequent blood and platelets transfusions. She had a normal karyotype study on the blood. The parents are first cousins and phenotypically normal. An older brother died of the same condition suggesting an autosomal recessive mode of inheritance. We think this constellation of clinical features is likely to represent a severe variant of Rothmund Thomson Syndrome. Other conditions including geroderma osteodysplatica and the severe autosomal recessive form of dyskeratosis congenital were also considered.

P0026. Mosaicism in genetic counselling procedure(Belarussian registry of chromosomal abnormalities)

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Mosaicism is cause of widespread spectrum of pathological features: mental delay, growth retardation, malformations, dysmorphic signs, hypogenitalism, decreased fertility, reproductive failure. Carriers need for exact cytogenetic status detection and prognoses.

We studied cytogenetical and clinical characteristics of 175 affected patients with mosaic chromosomal rearrangements (MChR) identified using GTG-banding analysis (lymphocytes). Mosaic abnormalities amounted to 6.7% of all number of aberrant karyotypes registered during 1983-2003 years (175/2619 cases). 48 different variants of MChR were detected.

Cytogenetical data. Spectrum of MChR included: mosaic aberrations of gonosomes -79.4%; autosomes -18.9%; combined mosaic cases -1.7%. Structural rearrangements amounted to 42.8% of all mosaic karyotypes (75/175 patients); sex chromosomes abnormalities prevailed among them -94.6% (71/75). Analyses demonstrated the presence of 2, 3 or 4 aberrant cell lines in single karyotype, including combinations of clones with normal and aberrant karyotype and abnormal clones only. Mosaic balanced karyotypes with inv(9) or inv(X) were registered in 4 cases (2%). 3 patients (1.7%) presented multiple aberrations.

Clinical data. The frequent mosaic chromosomal syndromes were: autosomal imbalance presented 24 cases (13.7%) of Down syndrome, 7 cases (4%) numerical, structural aberrations of autosomes 5; 8; 9; 13; 18; 22; sex chromosomes abnormalities include 110 cases (62.8%) of Turner syndrome, 5 cases (2.8%) - female with polysomy X, including pentasomy X, 9 cases (5.1%) of Klinefelter syndrome. Other cases showed rare combinations of numerical and/or structural MChR of gonosomes, autosomes and markers expressed by MCA/MR phenotype or hypogenitalism/reproductive failure features.

Spectrum of MChR and karyotype-phenotype correlations of rare variants will be presented.

P0027. Prenatal and postnatal MRI findings in Gomez-Lopez-Hernandez syndrome.

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Gomez-Lopez-Hernandez syndrome, or cerebellotrigeminal dermal dysplasia (MIM#601853), is a rare neurocutaneous disorder comprising cerebellar abnormalities, craniosynostosis, parietal alopecia, trigeminal nerve anesthesia, intellectual impairment and short stature. It has been reported in only ten patients, five of whom are Brazilian. Rhombencephalosynapsis is a rare cerebellar anomaly defined as fusion of the cerebellar hemispheres and agenesis of the vermis. It is usually a sporadic isolated finding, but has been consistently reported in Gomez-Lopez-Hernandez syndrome. We present the clinical and radiographic features of a 35-week premature Caucasian Australian male who had rhombencephalosynapsis and other brain abnormalities diagnosed on prenatal fetal MRI, initially identified on ultrasound. The diagnosis of Gomez-Lopez-Hernandez syndrome became apparent at six week postnatal review when parietal alopecia and abnormal skull shape was noted. Gomez-Lopez-Hernandez syndrome should be considered as a syndromic association of rhombencephalosynapsis. There may be a common pathogenetic mechanism.

P0028. A 13-year old true hermaphrodite male with 46, XX/47, XXY karyotype, positive SRY and azoospermia factor genes, diagnosed through a ruptured corpus luteum

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The vast majority of true hermaphrodite patients - characterized by the presence of both ovarian and testicular tissue - show ambiguous genitalia or severe hypospadias and almost all have vagina. The most common karyotype is 46,XX.

We report on a 13-year old boy, who presented with left inguinal hemorrhage after an accident while playing football. On clinical

examination, he had a left inguinal hernia, a palpable testis in the right semi-scrotum, normal male external genitalia and significant gynecomastia. During the operation for the left inguinal hernia, the left gonad and the adjacent tissue were removed for histological examination, which revealed the presence of a normal ovary, rich in follicles and a ruptured corpus luteum and a normal ipsilateral adnexa with semi-uterus. Endocrinological assessment postoperatively depicted high FSH levels, testosterone levels at the pubertal range and low estradiol levels. Cytogenetic analysis in peripheral blood lymphocytes revealed a 46, XX (70%)/47, XXY (30%) karyotype. FISH analysis of left gonad showed a 46, XX (60%)/47, XXY (40%) karyotype and molecular analysis verified the presence of SRY and azoospermia factor genes.

The importance of full histological, cytogenetic and molecular investigation in every single patient with sex differentiation disorders is highlighted by the constellation of normal male external genitalia with a 46,XX/47,XXY karyotype in this rare case of true hermaphroditism.

P0029. Czech dysplasia metatarsal type

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The authors report about four further patients of the recently described new bone dysplasia - *dominantly inherited pseudorheumatoid arthritis* that was diagnosed in eight members of one family. The patients of this report are not relative. They have a similar clinical history, the same distinctive phenotype and almost identical radiographic findings. The unique phenotypic hallmark is hypoplasia/dysplasia of the toes. The skeletal abnormalities are similar. They are localized predominantly in the spine, pelvis, hips and feet. They include a mild platyspondyly with irregularity of vertebral plates, narrowing of the joint and intervertebral disc spaces, rectangular shape of the lumbar spinal canal in the A-P projection, pelvic and proximal femoral dysplasia, and hypoplasia of the 3rd and/or 4th metatarsals. The disease is dominantly inherited with variable expressivity. The only major difference is absence of weather dependent articular pain that characterized the family of the previous study. All patients are Caucasians and originate from different parts of the Czech Republic. We conclude that patients in the previous study published under the name "Dominantly Inherited Pseudorheumatoid Dysplasia with Hypoplastic Toes" and those described in this report have the same disorder that is probably a quite common constitutional bone disorder in the Czech Republic. We propose this unique disease as a new nosological unit.

P0030. A French and Belgian collaborative study of 25 cases of Oral-Facial-Digital Syndrome Type 1: Clinical differences between patients with or without OFD1 mutation

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The oral-facial-digital syndrome type 1 or Papillon-Léage-Psaume syndrome belongs to the heterogeneous group of oral-facial-digital syndromes and is characterised by X-linked dominant mode of inheritance with lethality in males. Clinical features include facial dysmorphism with oral, tooth and distal abnormalities, polycystic kidney

disease and central nervous system malformations with wide inter- and intra-familial clinical variability. Mutations in the OFD1 gene are found in 50% of cases by direct sequencing. Large rearrangements and genetic heterogeneity could be postulated in order to explain this low mutation rate. A French and Belgian collaborative study collected 25 cases from 16 families and identified 11 novel mutations (9 frameshift, 1 nonsense and 1 missense mutation) by direct sequencing. We compared the phenotype of the 11 families with a pathogenic mutation with the phenotype of the 5 families with absence of mutation within the OFD1 gene in order to investigate which clinical features could suggest the presence or absence of an OFD1 mutation. There was no significant difference for the presence or absence of hypertelorism, cleft lip and palate, buccal frenulae, tooth abnormalities, digital abnormalities, corpus callosum agenesis and mental retardation between both groups. Conversely, we found that the presence of polycystic kidneys (χ^2 : 0.035) and short stature and the absence of lingual hamartomas (χ^2 : 0.041) could suggest the absence of an OFD1 gene mutation, although such data could not be useful at an individual level. It is interesting to note that short stature have been found only in the group without OFD1 mutation (4/9 cases).

P0031. An unusual case of Bannayan-Riley-Ruvalcaba syndrome with a mutation in the PTEN gene

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Bannayan-Riley-Ruvalcaba syndrome (BRRS) is characterised by macrocephaly, intestinal hamartomatous polyps, lipomas, speckled penis and mental retardation. Germline mutations in the tumor suppressor gene PTEN were described in patients with BRRS. Herein we report a case of BRRS with unusual clinical features and a novel mutation in PTEN gene. He was born after uncomplicated pregnancy with birth weight and length at the 90th centile, head circumference at the 75th centile. At the first observation (4.5 yrs) the patient has a height at the 90th centile, weight at the 50th centile and head circumference > +2 SD. He exhibited mild mental retardation and autistic features, joint hypermobility, macrocephaly, downward slanting palpebral fissures and multiple hyperpigmented macules on the shaft and glans penis. Colonoscopy revealed multiple intestinal lymphoid polyps, greater florid in the ileocecal region. The intestinal mucosa shows macroscopically numerous grey nodules and microscopically prominent large germinal follicles with polymorphous population of cells including B and T cell without any atypia. Mutation analysis revealed the presence of a missense substitution I135R in heterozygosity flanking to the phosphatase core motif at a position in which a I135V has been already described.

The peculiarity of our observation is related to the presence of intestinal lymphoid polyps, not yet described in BRRS, as well as the autistic features which have been rarely described in BRRS. Further studies are necessary to clarify the relationship existing between these features and the mutation described in our patient.

P0032. Cleft lip and palate and syndactyly in a father and son: new syndromic association?

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Cleft lip and palate is a common congenital malformation with an incidence of approximately 1 in 700 live births. It usually has a multifactorial etiology, with about 15% of cases being syndromic. This latter group is made up by a variety of syndromes, including Van der Woude syndrome as well as various chromosomal causes. We report on a father and son who were both born with bilateral cleft lip and palate and 3-4 syndactyly. The child was born at term after an unremarkable pregnancy and delivery to non-consanguineous parents. His father and mother were of Malaysian and East Indian descent, respectively. Aside from his father, the family history was non-contributory. He was noted at birth to have bilateral cleft lip and palate and was noted during our assessment to have partial 3-4 syndactyly of the left hand. There was a midline lower lip crease but no lip pits or bumps. The remainder of the medical examination was unremarkable. Development during

the first few months of life was normal. Our examination of his father revealed a repaired bilateral cleft lip and palate. There were no lip pits or bumps. There was bilateral partial 3-4 syndactyly. Chromosome analysis on the son revealed a 46, XY karyotype and negative FISH for 22q11. Hand X-rays on the father revealed no osseous abnormalities. We think that the association of these two findings may represent a previously unrecognized syndrome for which the inheritance pattern is likely autosomal dominant.

P0033. An unusual type of mixed gonadal dysgenesis associated with Müllerian ducts persistence, polygonadria and a 45,X/46,X,idic(Y)(p) karyotype

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Mixed gonadal dysgenesis (MGD) is a developmental anomaly in which most of the patients have a dysgenetic testis, a contralateral streak and a 45,X/46,XY karyotype. This entity involves an heterogeneous group of gonadal and phenotypic abnormalities with a wide clinical spectrum. Although the karyotype in these patients is 45,X/46,XY no genotype-phenotype correlation has been found to date. Müllerian ducts persistence (MDP) in MGD is rare; however, four patients with both entities and different karyotypes have been described. Here we present a newborn male assigned proband who was evaluated for ambiguous genitalia, two left testes, a right gonadal streak and Müllerian duct retention. The patient's original karyotype was 45,X[6]/46,XY[94] while the father's was 46,XY. Interestingly, the Y-chromosome in the patient was smaller than his father's Y, which had a very long Yq. PCR analysis identified all the Y-derived sequences tested in the father, while the patient had them all except the AZF b,c regions. FISH analysis of the paternal Y chromosome documented Yq paracentric inversion while in the patient an isodicentric chromosome with a Yp duplication was found, modifying the original karyotype to 45,X/46,X,idic(Yp). No mutations were observed in *MIS/MISRII* genes. Testicular ish karyotype showed X/XY dysgenetic testes and a 45,X streak gonad. We propose that the presence of the mosaic and the rearrangement of the Y chromosome in our patient could be responsible for the dysgenetic gonads leading to an abnormal function of *MIS* and androgens during early development.

P0034. Renal dysplasia and aortic coarctation: a report of four cases

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Aortic coarctation is a narrowing of the aorta, usually distal to the origin of the left subclavian artery. Renal dysplasia is defined as abnormal metanephric differentiation. Although untreated aortic coarctation may cause renal damage, most such cases detected prenatally are isolated or associated with Turner syndrome. We report four cases with renal dysplasia and aortic coarctation.

Case 1-Parents were first cousins of Pakistani descent. Fetal ultrasound showed severe oligohydramnios and bilateral multicystic, dysplastic kidneys. The pregnancy was terminated at 24 weeks. Autopsy confirmed the renal findings and identified an aortic coarctation. The karyotype was 46, XX.

Case 2- Fetal ultrasound showed oligohydramnios, abnormal renal tissue, and aortic coarctation. The child was delivered at 36 weeks gestation and lived 3 days. Autopsy revealed small, multicystic dysplastic kidneys, hypoplasia of the paraductal aorta and dysmorphic facial features. The karyotype was 46, XY and FISH for deletion 22q11.2 was normal.

Case 3- Fetal ultrasound showed oligohydramnios and poorly visualized kidneys. Postnatal investigation revealed aortic coarctation and bilateral hypodysplastic kidneys. The karyotype was 46, XY inv(18)(q22.1q23)mat and FISH for deletion 22q11.2 was normal.

Case 4- Fetal ultrasound revealed oligohydramnios, renal abnormalities and a VSD. Postnatal studies revealed a dysmorphic child with a

duplex right kidney with a dysplastic upper pole, a multicystic, dysplastic left kidney and a small aortic isthmus. The karyotype was 46, XX. Each case reported by us presented with oligohydramnios, dysplastic kidneys and aortic coarctation. This combination was reported only once in the literature and may represent a new syndrome.

P0035. Microphthalmia, sclerocornea, linear skin defect, abnormal cardiac conduction defect and no Xp22.31 deletion: are we dealing with a new syndrome ?

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Microphthalmia, sclerocornea and dermal aplasia syndrome is a rare X linked dominant entity. We report on a family with three affected daughters: The first daughter had congenital glaucoma and central leukoma of the left eye and Peters' anomaly. A second daughter had congenital diaphragmatic hernia, bilateral microphthalmia and died soon after birth. The third daughter was born with a linear facial skin defect, unilateral anophthalmia and sclerocornea. At the age of 6 months, she presented with junctional ectopic tachycardia which was ablated by catheterization. The two live affected daughters had a normal psychomotor development and no other malformation. The couple had 3 early spontaneous abortions, three healthy sons and one healthy daughter.

The constellation of eye malformations, skin defect (third daughter), diaphragmatic hernia (second daughter) and conduction defect (third daughter) fit the diagnosis of MIDAS or MLS syndrome.

High resolution karyotype was normal. Molecular analysis using polymorphic markers showed that marker DDX9985 was compatible with linkage to Xp 22.31, marker DDX7108 was recombinant.

The typical microdeletion of the known critical region for MIDAS/MLS was excluded in this family. Mutation analysis by direct sequencing of candidate genes located in the MLS critical region was negative in one of the member of the family

With the association of the rare entity of congenital junctional ectopic tachycardia, the question is whether we are dealing with a new syndrome.

P0036. Folate gene alteration is related to maternal cause of Down syndrome in meiosis II chromosome 21 nondisjunction cases

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The main origin of extra chromosome 21 in Down syndrome is usually maternal and occurs more frequently with advanced maternal age. In this study the parental origin, stage of meiotic error and folate gene alteration (C677T MTHFR) in 120 Iranian Down cases have been studied. Previous reports in that C677T MTHFR mutation have been studied as maternal cause of Down syndromes did not categorize Down cases according to their parental origin resulted mostly to a negative linkage. This is the first study to incorporate data of parental origin and timing of the chromosome 21 nondisjunction error into investigation of common mutations in methylenetetra hydrofolate reductase (MTHFR C677T) as maternal cause of Down syndrome in 120 mother of Iranian Down syndromes. The parental origin and stage of meiotic nondisjunction were determined in 100 Down children. Common mutation C677T of the MTHFR gene has been studied in mothers of Down syndrome and in 145 normal control mothers. Significant linkage was obtained only for mothers with maternal MII origin ($P<0.01$, $\chi^2>9.4$). All other categories showed negative linkage to the etiology of Down syndrome. This initial categorization of cases removes the sampling error and provides us with a better understanding of MTHFR gene mutation C677T as maternal cause of chromosome 21 nondisjunction. This finding shed light on etiology of Down syndrome and shows the importance of folate pathway gene alteration as a basic cellular failure to contribute significantly in the nondisjunction event of chromosome 21.

P0037. A diagnosis of LCHAD deficiency made 8 years after a child's death

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We report a family with 2 children. The first one, a female, was born in 1996. Her mother reported severe vomiting during pregnancy. At 3 months of age the girl fell ill and was hospitalized with the diagnosis of varicella encephalitis and myopericarditis. An inborn error of metabolism was suspected because of hepatomegaly, hypoglycemia and failure to thrive. At 6 months of age she fell ill with an acute respiratory infection and died suddenly of cardiorespiratory failure. The second child, a male, was born in 1997 after an uneventful pregnancy. The child developed well. At 6 years of age he fell ill with an acute respiratory infection and died suddenly from respiratory failure. Postmortem biochemical analyses of both children were done on blood from Guthrie cards. Acylcarnitine analysis suggested a diagnosis of LCHAD for the girl. The test was negative for the boy, but long sample storage may have resulted in degradation of acylcarnitines. It was decided to evaluate both parents' carrier status for a common mutation E510Q. Molecular studies (Dr. J.Zschocke, Institute of Human Genetics, Heidelberg, Germany) confirmed that both parents are heterozygous for this mutation. After these tests we could confirm the diagnosis of LCHAD deficiency for the girl 8 years after her death, but for the boy it is still uncertain whether he had LCHAD deficiency. Conclusion: these cases underscore the importance of comprehensive laboratory studies in patients with unclear deaths.

P0038. Analysis of Cx26 mutation: evidence for a Mediterranean ancestor for 35delG mutation

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Despite the fact that more than 22 genes have been identified in autosomal recessive non-syndromic deafness (ARNSD), a single gene, *GJB2*, accounts for a high proportion of the cases. One particular mutation, 35delG, was shown to have a high allele frequency in Mediterranean population. Recent studies indicate that the high frequency of this mutation arises from a founder effect rather than a mutational hotspot. We analyzed 70 Tunisian and 12 Moroccan families affected with ARNSD. Sixteen (Tunisia) and 6 (Morocco) families were homozygous for the 35delG mutation. One Tunisian family was compound heterozygous 35delG/291insA. In order to bring further evidence that the 35delG mutation arose in the Mediterranean population from a single mutational event on a founder chromosome, we analyzed the haplotype distribution in 31 unrelated individuals homozygous for the 35delG mutation (16 Tunisian, 6 Moroccan, and 9 Lebanese) and 116 unrelated normal hearing with no 35delG mutation (45 Tunisians, 41 Moroccan, and 30 Lebanese). Two microsatellite markers and one single nucleotide polymorphism were used for the haplotype analyses. Significant linkage disequilibrium (LD) between the 35delG mutation and the closest markers was observed. Our data suggest that the mutation is originated from one common ancestor in the Mediterranean population.

P0039. Mutation analysis of the TGFBI gene in families with hereditary corneal dystrophies from Ukraine

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Mutations in *TGFBI* gene encoding keratoepithelin are responsible for the group of autosomal dominant diseases of the cornea: granular (Groenouw type I), lattice type I, lattice type 3A, Reis-Bucklers and Avellino corneal dystrophies. In our study five previously reported mutations of the *TGFBI* gene: R124C, R124H, R124L (exon 4),

R555W, R555Q (exon 12) were analyzed using polymerase chain reaction followed by restriction digestion in 74 individuals from 30 unrelated families with different forms of corneal dystrophy from different regions of Ukraine. The R555W mutation was detected in patients from 5/10 families with suspected clinical diagnosis of granular corneal dystrophy. The R124C mutation was detected in 1 unaffected 10-year old individual and in patients from 11/18 families with lattice corneal dystrophy. The R555Q mutation was detected in 1 patient with clinical diagnosed Reis-Bucklers corneal dystrophy. As far as R124C mutation was detected in 1 patient with clinical diagnosed Reis-Bucklers corneal dystrophy we have concluded that this patient was misdiagnosed. These results show that *TGFBI* gene mutations analysis is important as well for early differential diagnosis of corneal dystrophies and genetic consulting in high risk families, as in future for development of effective preventive therapy.

P0040. Congenital aglossia with situs inversus totalis - A Case Report

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A 13-month old girl with difficult swallowing, growth and developmental retardation was referred to DEUGDC. The patient had a 7 year-old healthy sister and was the second child of non-consanguineous parents. Her birth weight was 3.300gr. and experienced aspiration pneumonia four times during her first months of postnatal history. Her physical examination revealed that, head circumference was 42cm. (<5p), height 60cm. (<5p) and weight 7.200gm (<5p). She has aglossia, high palate, dextrocardia, pectus excavatum, sacral dimple and pes planus. With ultrasonographic and radiological investigations situs inversus totalis was determined and her karyotype was 46,XX. The case was diagnosed as a rare syndrome named; Aglossia with situs inversus totalis. Although various genes which are thought to be responsible for the right and left axial development and misplaced body organs have been described previously, the etiology has been remained unclear. Description of the cases, comparison of the features with others and further detailed molecular investigations may lighten this complex subject.

P0041. Fragile X syndrome and alteration in folic acid pathway gene

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Fragile X syndrome happens due to the CGG repeat expansion and methylation of *FMR1* gene. Fragile X syndrome has an incomplete penetrance approximately 70% in male and 30% in female. To date less attention was to study important genetic factors that may play role in neuropathology and severity of physical characteristic of fragile X syndrome. Folic acid, has been prescribed in some fragile X cases for reduction of hyperactivity and attention deficit in prepuberty. Folic acid derivatives have important roles in DNA methylation and neural development and function as well as in synthesis of neurotransmitters and influence divers process in the CNS. Our hypothesis was to study impaired folate metabolism in fragile X patients as the first step to evaluate the linkage between folate metabolism dysfunction and methylation of *FMR1* gene. This is the first study to assess this hypothesis by comparing the frequency of three common mutations in C677T, A1298C methylentetra hydrofolate reductase (MTHFR) gene, in 40 male fragile X patients and 100 normal control males. All cases have been initially diagnosed with fragile X syndrome by molecular methods. The statistical analysis showed a significant linkage between C677T mutation with fragile X syndrome supported with a P value of 0.021 and chi-square of 7.719. However, no significant correlations were obtained for A1298C MTHFR mutation. Significantly high frequency of C677T MTHFR mutation may suggest effect of folate derivatives on neuropathology and methylation process and more investigations to perform on the role of folate metabolism in fragile X syndrome.

P0042. Screening of submicroscopic genomic aberrations in patients with X-linked mental retardation by high resolution array-CGH

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Subtle gains or losses of genomic regions seem to account for a substantial percentage (5-10%) of causes in patients with idiopathic mental retardation (MR). Therefore, we introduced the recently developed technology of array-CGH, which is able to detect cytogenetically undetectable microdeletions and -duplications, to identify novel genetic anomalies, and hence candidate genes, in X-linked MR patients. We have constructed an X chromosome-specific array containing 1875 clones resulting in an X-array with a theoretical resolution of 80 kb. Target DNA was spotted in duplicate on 3-D CodeLink slides. After extensive validation, differentially labeled genomic DNA probes of XLMR patients were co-hybridized on the X-array. We identified aberrations (0.3 - 1.5 Mb in size) in 10% of the patients. These were confirmed by real-time quantitation and 4 were not found in a control population suggesting a link with the MR phenotype. As an example, we report on a 1.5 Mb interstitial deletion at Xp22.3 that was detected in 4 unrelated MR patients with ichthyosis (XL1). Molecular analysis confirmed that this recurrent deletion occurred via non-allelic homologous recombination of the CRI-S232 repeat sequences, each of which contains a VCX gene. Moreover, the recombination site in all 4 patients was fine-mapped to a 1 kb repeat region present at the 3' end of the VCX-A and VCX-B genes, thereby deleting VCX-A but not VCX-B, providing additional evidence that VCX-A is the MR gene at Xp22.31. Additional data obtained with this X-array will be presented.

P0043. Association of TNF α promoter polymorphisms and cerebral palsy

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Cerebral palsy (CP) is a nonprogressive motor disorder caused by white matter damage in the developing brain. It is often accompanied with neurocognitive and sensory disabilities. The cause and pathogenesis of CP is multifactorial and continues to be poorly understood. Chorioamnionitis, clinical silent or manifest, has been reported to be a risk factor for CP both in term and preterm infants. TNF α is a cytokine produced by activated monocytes and macrophages, which play a key role in the inflammatory response. TNF α gene is mapped to chromosome 6p21.3 and a large number of polymorphisms of its promoter, called "high-production" polymorphisms, have been described. Increased TNF α levels in peripheral blood in premature and close-to-term birth have been found to associate with the development of CP. The aim of our study was to estimate allelic frequency for four promoter region SNPs in TNF α gene, -238, -308, -857 and -1031 in the children with the CP.

DNAs obtained from peripheral blood of 40 CP patients and 150 unrelated healthy volunteers were genotyped for the TNF α -238, -308, -857 and -1031 SNPs using real-time PCR TaqMan[®] SNP genotyping assays. There was statistically significant correlation ($p<0.05$) between cerebral palsy and expression affecting allele variants of TNF α , -308A and -857T. The association between these polymorphisms and cerebral palsy has to be investigated in the future studies.

P0044. Analysis of the 12S ARNr gene : Detection of the A1555G mitochondrial mutation in a Tunisian family affected with non syndromic hearing loss and novel polymorphisms in Tunisian population

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Mitochondrial mutations especially in the mitochondrial 12S rRNA and the tRNA genes have been found to be associated with both syndromic and nonsyndromic hearing loss. In particular, the A1555G mutation in the 12S rRNA gene has been associated with non-syndromic deafness

in many families of different ethnic backgrounds.

Since mitochondrial mutations have not been yet searched in Tunisian deaf patients, we have attempted to explore the 12S ARNr and the ARNr^{Ser(UCN)} genes in our sample.

We have studied 125 patients affected with hearing loss and belonging to 114 Tunisian families for whom no nuclear mutation responsible of hearing loss have been identified.

Searching for the A1555G mutation have been performed by PCR-RFLP. The entire mitochondrial 12S ARNr gene have been explored by direct sequencing.

The A1555G mutation have been identified in one out of the 114 families and not in 100 normal individuals. After direct sequencing of the mitochondrial 12S RNA gene, we have detected known polymorphisms at nucleotides 709, 710, 750, 769, 825, 930, 1018, 1048 and 1438 in patients and normal individuals. We have also detected three novel polymorphisms in the same gene.

The mutational screening of the mitochondrial ARNr^{Ser(UCN)} gene shows the absence of A7445G, 7472insC, T7510C, T7511C and T7512C mutations in this gene in the 125 patients and the 100 controls analysed.

We report here the first mutational screening of the mitochondrial 12S ARNr gene in Tunisian population which describes the second family harbouring the A1555G mutation in Africa and reveals three novel polymorphisms.

P0045. Mutation 35delG in the gene GJB2 and hereditary pre-lingual deafness among the Russian population.

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GJB2 is the gene which encodes the gap junction protein connexin 26. A homozygotic carrier of the mutation 35delG in this gene is to be a cause for recessive non-syndromic sensorineural hearing loss or deafness in the pre-lingual period of the Caucasoid population. Investigation conducted among 44 patients with pre-lingual sensorineural hearing loss or deafness in St-Petersburg, 35delG/35delG genotype was found in 21 patients (47.7%), 35delG/wt in 7 patients (16%). At the mean time early childhood deafness associated with 35delG mutation was found more frequently among the children with healthy (heterozygotic) parents (43.8%) and less frequently among the children with deaf (homozygotic) parents (15%). Interestingly in the control group with 238 healthy donors, 14 (5.9%) were heterozygotic mutation carriers. This shows the highest level of heterozygosity among all Caucasoid populations.

P0046. Creating a Path for the Acceptance of Microarray-Based Genetic and Genomic Tests in the Clinical Laboratory

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Few inventions in the past fifteen years have had as much impact on genetic and genomic research as microarray technology. This technology has captured the imaginations of clinical and laboratory researchers and made possible studies heretofore unachievable. The scope and breadth of the studies enabled by this technology is changing the practice of laboratory and clinical research and has led to the introduction of array-based diagnostic tests in the clinical laboratory. Whole genome analyses promise deeper insight into biological processes, biological reporters and discovery of new biomarkers and the volume of genetic and genomic data places pressure on scientists and clinicians to design experimental and analyses strategies that leverage the integration of clinical and molecular information. This new era of clinical genetics and genomics places increased emphasis on collaboration and teamwork including a significant focus on well-defined tissue banks and clinical databases. At the center of this paradigm shift is a requirement for the tools necessary for measuring technical performance of the platforms used for data collection. Reporting reliable information of known quality is key to the emergence of microarrays in toxicogenomics, pharmacogenomics, and as diagnostic devices in clinical medicine. Success in moving microarray technology into clinical and diagnostic laboratories requires building a research and development foundation in multiple areas including standardization across industry, development of clinical guidelines via accredited

processes, generation of validation data, diagnostics-suitable platform delivery and achievement of regulatory approval. Examples of key successful projects and partnerships will be shared.

P0047. Clinical variability in a Noonan syndrome family with PTNP11 gene A118G mutation

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Noonan syndrome (NS) characterized by craniofacial anomalies, short stature, congenital heart defects, cryptorchidism, skeletal and other organ malformations, is an autosomal dominant disorder with different mutations of PTNP11 gene in 60% of cases. We report on a family with NS transmitted through 3 maternal generations. All five affected members presented with the same morphological characteristics but different organ involvement. The index case a 5 years old girl with height in the 3rd percentile is the product of a twin IVF pregnancy. Her phenotypic characteristics, common in all affected members, include: coarse and triangular - shaped face, hypertelorism, down- slanting, prominent eyes with epicanthus, low - set posteriorly angulated ears with thickened helices, nasal tip bulbous, big mouth with arched upper lip, short webbed neck, pectus excavatum and scoliosis. At the age of 1 year she was operated for pulmonary stenosis as was her twin sister who died after the operation. The 39 years old mother started menstruation at the age of 16 and has a history of bleeding diathesis. Maternal uncle 30 y.o. has a heart murmur. The grandmother died at the age of 30 years because of a heart attack. The molecular investigation revealed a maternally transmitted heterozygous missense mutation A118G in exon 3 of PTNP11. The infrafamilial variability of NS makes it essential for proper genetic counseling, so that first degree relatives, even with atypical phenotype, should be screened for PTNP11 gene mutations.

P0048. The Neurofibromatosis Type 1 - Multisystem Disease Requiring Multidisciplinary Approach

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The Neurofibromatosis type 1 (NF1) is one of the most common single gene diseases (the expected incidence is 1:3000). Around 50 % of cases represent fresh mutations, the carriers of this mutation have 50 % risk for their offspring without possibility of prediction of the age of manifestation and disease progression. The NF1 disease is characterized by extremely high interfamilial and intrafamilial variability.

There was implemented a database of 200 records of patients suffering from NF1 in the Neurogenetic Centre of the Institute of Biology and Medical Genetics, Child Neurologic Department and Neurologic Department of the 2nd Medical School, Charles University.

By the family history analysis, the 40% cases have been identified as fresh mutations, in the 1/3 of cases the serious degree of the disease has been detected.

The haplotype analysis has been carried out in 29 families. The complex investigation methodology in the patients with a NF1 has been verified. Both the molecular cytogenetic (FISH) analysis and the efficient and reliable molecular genetic diagnostics (DHPLC) have been introduced with the goal to detect causal mutations.

Several complicated cases which document the necessity of the multidisciplinary approach will be demonstrated.

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P0049. Frameshift mutation in ZHX1B gene in a Croatian girl with Hirschprung disease, mental retardation and epilepsy (Mowat Wilson syndrome)

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Background: Mowat Wilson syndrome (MWS) includes distinctive facial appearance, intellectual impairment, seizures and other malformations such as Hirschprung disease, congenital heart defects and agenesis of the corpus callosum. It is caused by heterozygous deletions or truncating mutations of the ZFX1B gene on chromosome 2q22.

Case report: We present a 13-years-old Croatian girl born to healthy and unrelated parents. Because of short segment Hirschprung disease emergency colostomy was performed at 2nd day of life. Dysmorphia, hypotonia and failure to thrive have also been observed. Since the 1st year of life the antiepileptic therapy was introduced because of seizures and pathologic changes on EEG. Brain MRI showed hypoplastic corpus callosum. At the age of 13 years she was normocephalic, but with severe mental delay, happy disposition and distinctive dysmorphic features: sparse hair and eyebrows, sloping forehead, telecanthus, broad nasal bridge, large ears, pointed chin and long fingers and toes. We suspected clinically to the possible mutation in ZFX1B gene and de novo frameshift mutation 1352delC (exon 8) in the heterozygous state was found. It corresponds to the deletion of the nucleotide C 1352 of the gene coding sequence and results in a frameshift creating a STOP codon at position 453 of the protein.

Conclusion: All children with syndromic Hirschprung disease should be tested for mutation or deletion in ZFX1B gene. The early diagnosis of MWS enables adequate neuropediatric follow-up because in majority of children with MWS the epilepsy could appear during the first year of life or later.

P0050. OPD2/Melnick-Needles syndrome with occipital encephalocele.

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Oto-Palato-Digital syndrome type 2 and Melnick-Needles syndrome both are X-linked dominant conditions with lethality in males. We report on a male patient with an additional feature. At 18 weeks of pregnancy, multiple congenital malformations (sharply bowed femora, occipital encephalocele) were found on ultrasound. The parents refused any further investigation and wished to carry on with the pregnancy. At 38 weeks of pregnancy, a stillborn macerated boy was born. Multiple malformations were noted. The skull was large with a large occipital encephalocele. The face was dysmorphic (hypertelorism, flat nose, large tongue). The palate was closed. There were elbow contractures with webbing, missing terminal phalanges of both thumbs, small middle phalanges of all fingers and small nails. There were sharply bowed femora, completely absent halluces and syndactyly of toes 4 and 5 bilaterally. There was a small penis with hypoplastic scrotum and absent testes. On X-rays the clinically noticed abnormalities were confirmed. Moreover the boy had radiologically malformed clavulae and scapulae, high vertebral bodies and a markedly advanced bone age. Chromosomes were normal, 46XY. Internal organs were normal although the bladder was reported as large. The testes were not found.

The combination of congenital malformations fits in the OPD2/Melnick-Needles spectrum. However, occipital encephalocele was never reported in these conditions. Sequencing of exon 22 of the FLNA gene did not reveal a mutation. Further mutation analysis is in progress

and will be reported. Whether occipital encephalocele is an additional feature or a coincidental finding depends on further case reports.

P0051. Long-term follow-up of patients with Kabuki syndrome in Taiwan

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Kabuki syndrome (KS), also called Niikawa-Kuroki syndrome, is a rare congenital disorder of unknown etiology. Most KS cases occur sporadically. Five major criteria delineate KS namely postnatal short stature, skeletal anomalies, moderate mental retardation, persistent fetal pads on the fingers, and a characteristic facial dysmorphism. Long-term follow-up of twelve individuals with KS in Taiwan indicates a broad spectrum of neuro-psychiatric or metabolic dysfunction and mental ability. The incidence of seizure (generalized, complex partial, or atonic seizure) is 50% (6/12). Seizures were well controlled in 5 cases. Retarded mentality was noted in all child patients except for in a 40-year-old man. Hearing impairment was found in 8 patients probably after recurrent otitis media or microtia. Growth hormone deficiency was detected in two KS girls. Recombinant human growth hormone were administered with a favorable outcome in both cognition and body height. Attention deficit disorder and hyperactivity occurred in 9 patients. Among these patients, a boy developed depressive psychosis after the age of 12 years. Obesity with relative short stature were common especially after the age of 8 years. One 13-year-old girl had obstructive sleep and fatty liver. She developed type 2 diabetes two years later. For KS patients, a long-term monitoring and evaluation of their neuro-psychiatric status, hearing function, and endocrinologic-metabolic profile is recommended.

P0052. Polymorphism of metabolic genes and susceptibility to occupational chronic toxic hepatitis

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In this study we investigated genetic polymorphisms of five metabolizing genes and their association with toxic hepatitis. PCR-RFLP was used to genotype the cytochromes P4501A1, 2D6, 2E1 (CYP1A1, CYP2D6, CYP2E1), microsomal epoxide hydrolase and the N-acetyltransferase 2. We recruited 73 patients with toxic hepatitis, 163 workers "groups of risk" on development of a toxic hepatitis, 94 healthy workers and 335 unrelated healthy control subjects. The controls were matched to the cases by sex, age as well as the heptyle and ethylebenzene-styrene exposure duration. No significant association was found between the control and petrochemical workers when CYP1A1, CYP2D6, CYP2E1, EPHX1 genotypes were included in the analyses. Among workers was observed the increasing frequency of a combination NAT2*4/*4 genes NAT2 compared with control group. Among the patients with a toxic hepatitis are established genetic markers of predisposition to development of the disease: Ile/Val gene CYP1A1, Tyr/His gene EPHX1; combinations NAT2*4/*7 genes NAT2; and as slow phenotype microsomal epoxide hydrolase; combinations of genotypes Ile/Val/C1C1 of genes CYP1A1 and CYP2E1; combinations of slow phenotypes microsomal epoxide hydrolase and N-acetyltransferase 2. Our results suggest that genotype Ile/Ile of gene CYP1A1; genotype Tyr/Tyr of gene EPHX1; and as a normal phenotype microsomal epoxide hydrolase; a combination of normal genotypes of genes CYP1A1 and CYP2E1; a combination of normal genotypes of genes CYP1A1, CYP2E1, CYP2D6 and a normal phenotype microsomal epoxide hydrolase are protective variants. This study demonstrates a significant combined effect of genes on the predisposition of pathology at workers exposed to heptyle and ethylebenzene-styrene.

P0053. Case report: child with mosaic trisomy 3

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We report a case of mosaic trisomy 3 in a one year old boy. He is the fifth child of normal, healthy parents with normal karyotypes. Cytogenetic analysis of blood lymphocytes was done for evaluation of dysmorphic features. Chromosome analysis of blood cells with G - banding

showed only 47, XY,+3 karyotype while analysis of skin fibroblasts revealed mosaic karyotype - 47, XY, + 3 / 46, XY. Fluorescence in situ hybridization (FISH) analysis using an alpha satellite chromosome 3 probe performed on skin fibroblasts demonstrated two signals in 198 nuclei (46,XY) and three signals in 2 nuclei (47,XY, + 3). This result confirms the diagnosis of low level trisomy 3 mosaicism. The propositus was born after a full term pregnancy. Several dysmorphic facial features were noted: caratacts of both eyes, cleft upper right lip and complete cleft of the palate. Heart ultrasound showed ASD secundum. Brain US and CT showed periventricular hypoxia with cystic leucomalacia. Genetic counselling in such a family can be difficult. As both parents in the present case had normal karyotypes, the risk seems to be extremely small but it can also be further minimized if the next pregnancy is properly supervised. Additional cases of postnatally diagnosed mosaicism for rare trisomies are necessary for more accurate assessment of the significance of our cytogenetic and clinical findings.

P0054. Quantitative analysis of *SMN1* and *SMN2* genes based on DHPLC: A highly efficient and reliable carrier-screening test

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Autosomal recessive spinal muscular atrophy (SMA) is a common, fatal neuromuscular disease caused by homozygous deletion/conversion of the *SMN1* gene in more than 95% of patients. However, a highly homologous *SMN2* gene exists in the same chromosome, interval, centromeric to *SMN1*, and hampers detection of *SMN1*. We present a new, rapid, simple, and highly reliable method for detecting the *SMN1* deletion/conversion and for determining the copy numbers of the *SMN1* and *SMN2* genes by denaturing high-performance liquid chromatography (DHPLC). We analyzed *SMN1*/*SMN2* gene exon 7 deletion/conversion by DHPLC. Eighty patients with spinal muscular atrophy lacking the *SMN1* gene in seventy-five families, 500 control individuals from the general population and the family members were analyzed. By DHPLC analysis, we could detect the SMA-affected cases efficiently just by recognizing an *SMN2*-only peak. Furthermore, after specific primer amplification and adjustment of the oven temperature, all of the SMA carriers with an *SMN1*/*SMN2* ratio not equal to one could be identified unambiguously by this simple and efficient detection system. To calculate the total *SMN1*/*SMN2* gene dosages further, we developed a specific multiplex competitive PCR protocol by simultaneously amplifying the *CYBB* gene (X-linked), the *KRIT1* gene (on chromosome arm 7q), and the *SMN1*/*SMN2* gene ratio by DHPLC. By applying this technique, we could successfully designate all of the genotypes with different *SMN1*/*SMN2* gene copy numbers, including equal and unequal amounts of *SMN1* and *SMN2*. We demonstrated that DHPLC is a fast and reliable tool for detection of carriers of SMA.

P0055. Rapid detection of large deletions and duplications in Duchenne muscular dystrophy carriers by denaturing HPLC coupled with multiplex PCR

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Duchenne muscular dystrophy (DMD) with X-linked recessive inheritance is the most common neuromuscular disease in children. One-third of the affected patients come from de-novo mutations without family histories. About 65% of the cases involve large intragenic deletions, and about ~6% involve large intragenic duplications of one or more exons in the dystrophin gene. We describe the first use of denaturing HPLC (DHPLC) to identify the DMD carriers. We used multiplex polymerase chain reaction (PCR) to amplify nineteen exons to analyze the dystrophin gene. We optimized multiplex PCR with DHPLC for rapid diagnosis of eleven DMD affected males, 23 obligate carriers and non-carrier from DMD families, and 50 individual controls with high sensitivity and resolution. By applying this technique, we could successfully calculate the ratio of test exon / reference exon in the dystrophin gene. Our study has analyzed several families for the dystrophin gene deletions and has shown that multiplex PCR/ DHPLC

analysis can be an effective and direct method for establishing the DMD carrier and noncarrier status of females. These results provide a strong support that DHPLC with multiplex genotyping is an efficient and reliable tool for high throughput diagnosis of DMD. We demonstrated that DHPLC can be used to analyze the relative gene dosage from large deletions in the dystrophin gene. It is a fast, reliable and powerful tool for the identification of DMD carriers by DHPLC coupled with multiplex PCR. We believe the described method will be suitable for routine clinical application in the future.

P0056. Study of CF Mutation in the CFTR Gene of Iranian patients

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Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator gene and characteristically leads to prominent lung and pancreatic malfunctions. Cystic fibrosis is the most common autosomal recessive disease in the Caucasian population with a prevalence estimate of 1 in 2500 to 3300 live births. About 1000 mutations have been detected so far. A few mutations are spread worldwide, the majority are being "private" mutations.

In this study Seventy unrelated Iranian CF families were screened for the presence of five common mutations (F508, G542X, W1282X, G551D, N1303K) using ARMS PCR. Exons 4, 7, 10, 11, 13, 20, 21 of the CFTR gene were tested for the presence of any possible mutation by SSCP and sequencing method. This study resulted in the identification of 28 per cent of all CF alleles. DelF508 was detected in the 18 per cent of the tested alleles.

Our findings suggest heterogeneity in the Iranian population, stressing the need to draw attention to sequence analysis in order to find population-specific mutations.

P0057. del5p/dup5q in a 'Cri-du-chat' patient without parental chromosomal rearrangement: reminding gonadal mosaicism

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We report a full term male infant born to nonconsanguineous parents who presented at birth with multiple congenital abnormalities and distinctive high-pitched mewing cry. He was 2350g in weight (<3%), 46 cm in height (<3%), and had 31.5 cm head circumferences (<3%) at 11th day. The physical examination revealed round face, hypertelorism, epicanthal folds, downward slanting of the palpebral fissures, micrognathia, retrognathia, bilateral incomplete helical architecture of horizontally asymmetric low set ears, preauricular skin tag anterior to the left auricular region, long philtrum, bilateral simian line, bilateral hallux-valgus deformity, small muscular ventricular septal defect and secondary atrial septal defect.

Chromosome study from the peripheral blood lymphocytes showed to be normal 46,XY karyotype. However, fluorescence in situ hybridization (FISH) studies using a cri-du-chat probe (5p15.2 and 5p15.3, 5qter: CP5102-DC, Appligene) demonstrated loss of cri-du-chat critical region. He also had terminal 5q region duplication. This finding was also verified using comparative genomic hybridization (CGH), which revealed a loss of 5p14 → pter and a gain of 5q33 → qter. Cytogenetic and FISH analysis were carried out for each parents to describe the derivative chromosome, but surprisingly, pericentric inversion or any other balanced rearrangement were not found.

In this report, we describe the first cri-du-chat case with partial monosomy of the short arm and partial trisomy of the long arm of the chromosome 5, but without any parental balanced chromosomal rearrangement. These results suggested a possibility of gonadal mosaicism for pericentric inversion of chromosome 5 in one of his parents.

P0058. Is p53 gene polymorphism associated with multiple sclerosis in Iran?

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To investigate the relationship between p53 codon 72 polymorphism and risk for multiple sclerosis (MS), we collected samples from MS patients and healthy population from different region of Iran with different ethnicity groups (Fars, Mazandarani and Turk). The p53 Pro72Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) in 207 healthy controls and 60 MS patients. Among the patients and healthy subjects with Fars, Mazandarani and Turk ethnicity, the genotype frequency of p53 Pro72Arg were 23.3% and 34.8% for Arg/Arg, 76.7% and 45.9% for Arg/Pro, 0% and 19.3% for Pro/Pro, respectively. Significance differences were found for p53 allele distribution among patients and healthy individuals. Our finding suggested that the frequency of Arg/Pro genotype is more than healthy Iranian population and p53 genotype may play an important role in developing multiple sclerosis among Iranian population.

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P0059. A counseling dilemma posed by a fetus with a split-hand/-foot malformation and a father with a short 3rd toe

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Split-hand / split-foot malformation (SHFM) occurs in isolation and as a feature of many syndromes. After birth, the various conditions can usually be distinguished clinically.

SHFM was detected in a male fetus on routine ultrasound at 18 weeks gestation. A central ray defect was noted in three extremities. One hand had only four metacarpals. The other had five metacarpals, but only four fingers. The two ulnar fingers were webbed bilaterally. One foot had webbing of the 1st and 2nd toes. There were no other abnormalities noted and the fetus was appropriate in size for gestational age.

Examination of the mother was normal. The father had a normal physical exam except for a short 3rd toe unilaterally.

The family history was considered unremarkable. The pregnancy was terminated because of the possibility of a syndromal form of SHFM with cognitive impairment.

Subsequent mutation analysis of the p63 gene from leukocytes obtained by cordocentesis revealed heterozygosity for a mutation in exon 5 (R204Q). This mutation has previously been described in association with the EEC (Ectrodactyly-Ectodermal dysplasia-Clefting) syndrome.

Mutation analysis of peripheral leukocytes in the father revealed heterozygosity for the same mutation (R204Q). No evidence supporting mosaicism was found on analysis of DNA from a buccal smear. On reassessment, it surfaced that the father had weak dental enamel and recurrent eye infections as an adult due to stenotic tear ducts.

Our case underscores the heterogeneity of phenotypes associated with p63 mutations and the usefulness of mutation analysis in counseling for SHFM.

P0060. Delayed puberty and severe osteoporosis in genitopatellar syndrome

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Genitopatellar syndrome (OMIM 606170), first described by Goldblatt et al. in 1988, is a rare disorder with characteristic facial features, genital anomalies, absent patellae, flexion contractures, microcephaly, renal anomalies, mental retardation, poor outcome and possible autosomal recessive inheritance. At present, eleven cases have been reported. We describe an adolescent Finnish female with findings consistent with genitopatellar syndrome. She is the second child of her healthy, unconsanguineous parents. She has microcephaly, broad nasal bridge,

midfacial hypoplasia, high palate, round cheeks, long chin, flexion contractures of the hips and knees, absent patellae, clubfeet, limited movement of shoulders, brachydactyly with prominent joints, scoliosis, hypertrophic clitoris, dysplastic kidneys with mild hydronephrosis, hypoplastic corpus callosum and severe mental retardation and motor delay. The karyotype is normal examined from peripheral blood and fibroblasts. Telomeric deletions and Smith-Magenis sdr have been ruled out with FISH.

At age 14 years and 9 months she has hypothyroidism (TSH 17 mU/l, free T4 7.1 pmol/l., TRH- and ACTH-tests consistent with intact hypothalamus-hypophysis-thyroid and hypophysis-adrenal axes) and no signs of puberty (Tanner stage 1). The gonadotrophin values are prepubertal (LH <0.1 U/l and FSH 1.6 U/l). The growth hormone secretion is normal based on appropriate S-IGF-1 concentration. She also has abnormal bone structure and severe osteoporosis (gender and bone age-adjusted lumbar bone mineral density is -4.8 SD) with a history of seven bone fractures.

Being the oldest reported patient with genitopatellar syndrome, our patient is the first one showing abnormal endocrine findings including a lack of puberty and symptomatic osteoporosis

P0061. A furher case of Costello syndrome

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Costello syndrome is a rare genetic disorder of which underlying molecular defect has not been defined. The patients with Costello syndrome (CS) have short stature, mental retardation, coarse facial features suggestive of a storage disorder, loose soft skin with deep palmar and plantar creases, joint laxity, increased skin pigmentation, skeletal abnormalities, cardiac problems, increased predisposition to solid tumors.

We describe an 8 year-old boy who had characteristic features of Costello syndrome and showed feeding problem in late childhood period.

The patient was born to noncansanguineous parents at term after an uneventful pregnancy. Birth weight was 1900 gm. He was brought to hospital for the first time at 5 years of age because of redundant skin and developmental delay. He was diagnosed to have cutis laxa. At 8 years old he was hospitalized due to anorexia and weight lost. He had mental retardation but he was quite social. He was noted to have coarse facial features with typical lips, blue sclera, high arched palate, dentalabnormalities, abnormal hair curve, redundant skin, deep palmar and plantar creases, joint laxity, dark skin. Laboratory tests including CBC, urinalysis, blood electrolytes and glucose levels, VMA in urine were normal.

He was reluctant to eat his normal daily diet. During hospitalization period he was given special formula containing high protein and kalori. He started to gain weight in a short period.

In Costello syndrome feeding can be aproblem even in older ages and special diet can be required.

P0062. Clinical and molecular characterization of a novel autosomal recessive Norrie-like syndrome

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Norrie syndrome is a rare X-linked neurodevelopmental disorder mainly characterized by blindness resulting from retinal malformations, opacity of the lens and atrophy of the iris. In some cases, children may have varying degrees of mental retardation and hearing loss.

We here report the clinical findings of two brothers and a female cousin born from distant consanguineous marriages who had bulboptysis, mental retardation, and a mild myopathy. The eye findings were almost identical to those found in patients with Norrie syndrome and characterized by corneal opacity, cataract, iris atrophy, retinal detachment, microphthalmia. progressing to bulboptysis. EMG analysis revealed signs of slow progressive myopathy at the lower

and upper limbs which correlated with the muscular atrophy mainly involving the lower limbs. All three patients had mild mental retardation. We excluded mutations in the Norrin gene by direct sequence analysis and propose that the disease in this family is a novel autosomal recessive Norrie-like syndrome.

As a first attempt to identify the molecular basis of the disease, we performed a genome-wide linkage analysis in the family using polymorphic microsatellite markers and found a statistical significant linkage to a chromosomal region that has been not yet described for eye disorders complicated with mental deficiency and muscle involvement. Refinement of the critical region and testing of candidate genes located in the region is currently performed and will be presented.

P0063. A family of ulnar-mammary syndrome with three affected members

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Ulnar mammary syndrome is a rare autosomal dominant disorder characterized by post axial ray deficiency, dental abnormalities, mammary and apocrine gland hypoplasia, genital developmental anomalies. Affected individuals may show variable phenotypic expression changing from full characteristics of the syndrome to only post axial ray defect. Recently mutations in the TBX3 gene have been demonstrated to cause ulnar mammary syndrome.

We describe a family of ulnar mammary syndrome with three affected members. The proband was 2 year-old girl who had 6 fingers on the right hand, 7 fingers on the left hand. She also had syndactyly of the 4th,fifth and 6th fingers on the left hand. A skin appendage with a small underdeveloped nail was noted as the 7th finger. She had clinodactyly and absent distal flexion crease of the fifth finger and 6 toes on both feet. Canines of upper jaw were absent. She had scapula lata, absent nipples, truncal obesity, pes planus. She was operated due to hydrometacolpos in neonatal period.

The son of maternal aunt had postaxial polydactyly on both hands. The son of maternal grandfathers brother had postaxial polydactyly in both feet.

P0064. Detection of subtelomeric chromosomal rearrangements by FISH analysis in cases with idiopathic mental retardation and dysmorphic features

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Mental retardation (MR) has a 2-3% frequency in general population. The etiology is remains unknown in more than half of the cases. In recent years, technical improvement of molecular cytogenetics has proved that cryptic chromosomal rearrangements are a significant cause of idiopathic MR.

Subtelomeric regions of the chromosomes are enriched with CpG islands and are believed to have the highest gene density in the genome. The cryptic subtelomeric rearrangements resulting in gene-dosage imbalance might represent a significant cause of idiopathic MR and dysmorphic features with normal conventional karyotypes.

As the telomere regions of the chromosomes are G-band negative and morphologically similar, the abnormalities in these regions are thought to be particularly difficult to detect by using conventional cytogenetic methods. The cryptic rearrangements of distal ends of the chromosomes that can not be detected by conventional cytogenetic methods have been proved by Fluorescence In Situ Hybridization (FISH) using chromosome-specific subtelomeric probes.

In our study, 20 cases with idiopathic MR and dysmorphic features with normal conventional karyotypes were investigated by FISH using subtelomeric probes. Subtelomeric chromosomal rearrangements were detected in 3 of 20 patients. One patient had a familial cryptic unbalanced translocation between subtelomeric regions of the chromosome 5p and 15q. Another patient had a de novo cryptic unbalanced translocation between subtelomeric regions of the

chromosome 18p and 22q.

In conclusion, the detection of subtelomeric chromosomal rearrangements are of great importance in offering genetic counseling and prenatal diagnosis in both familial and sporadic cases with idiopathic MR and dysmorphic features.

P0065. A family of type I BPES with 5 affected members

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Blepharophimosis-ptosis-epicanthus (BPES) syndrome is a rare genetic condition characterized by short palpebral fissures, ptosis of eyelids, inverted inner canthal fold between upper and lower lids and lateral displacement of the inner canthi. It is transmitted by autosomal dominant inheritance and most cases are sporadic. More than 53 mutations in FOXL2 gene have been reported to be responsible for the BPES. Type I in which the syndrome is transmitted only through males due to the female premature ovarian failure, whereas type II can be transmitted by both affected females and males. Type I is associated with menstrual irregularities and infertility. Here we present a family of BPES with 5 affected members. The proband who was a 15-year-old boy was referred to genetics outpatients clinic for genetic counseling. He showed all characteristic findings of the syndrome. Family history revealed 5 more affected cases of which 3 were operated due to severe blepharophimosis. Karyotypes of all cases were normal. We also point out the importance of distinction between two types of BPES in genetic counseling of female patients.

P0066. Spondylocostal dysplasia in a three generation family with 4 affected members

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Jarcho-Levin syndrome is an eponym that has been used to describe a variety of clinical phenotypes consisting of short-trunk dwarfism associated with rib and vertebral anomalies. Molecular, clinical and radiological data have allowed further characterization of these phenotypes. Based on these findings, Jarcho-Levin syndrome has been divided into two types as spondylothoracic dysplasia and spondylocostal dysplasia.

We report a 4.5-year-old boy showing typical features of spondylocostal dysplasia. He referred to us with short length, short neck and malformed trunk.

The proband was a 4.5 year-old boy with typical features of spondylocostal dysplasia such as short stature, short neck, chest deformity. There was no parental consanguinity. He has a healthy 8 years old sister and his mother experienced 2 abortions at 10 weeks of gestation. Family history revealed three more affected members with variable expressivity in paternal side. Chest X-ray of the proband showed fusion defects of the ribs, bifid costa and vertebral deformities. He also had spina bifida, platyspondyly, scoliosis and vertebral fusions. We present our case as an example of autosomal dominant inheritance of Spondylocostal Dysplasia. We also point out the importance of distinction between two types in genetic counseling of Jarcho-Levin Syndrome patients.

P0067. Is empty Sella Syndrome the main cause of pituitary dwarfism?

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Introduction: The empty sella syndrome is a relatively new entity, developed in parallel with improved imaging techniques (CT and MRI). Empty sella refers to the absence of the pituitary gland on CT or MRI. The turcic sella is partially or completely filled with CSF. Empty sella syndrome is the pathologic variant of the imaging-described empty sella.

Objectives: A review of current knowledge in empty sella syndrome and its association with pituitary dwarfism. **Material and methods:**

We observed a series of 13 children with pituitary dwarfism over a 10-year period. All patients were diagnosed with pituitary dwarfism based on clinical features (including somatometrie) and confirmed by dosage of serum growth hormone level. Of these, nine patients also had brain MRI done. **Results:** From the nine patients, eight had empty sella on the MRI. This very height percent (89 %) is most frequently than found in literature (5-58%). MRI is the elective imagistic method, other methods like X-rays, head ultrasound or CT may not be relevant. One of these eight cases was selected for presentation: it showed an association of empty sella syndrome, pituitary dwarfism and polydactyly. The association of pituitary dwarfism and polydactyly has been communicated in only four cases in the literature. **Conclusions:** The association of the empty sella syndrome and pituitary dwarfism in our series is most frequently that in literature; defining it will improve the knowledge of the etiology and pathogenesis of pituitary dwarfism. The association with polydactyly is very rare and could be syndromic or random.

P0068. Investigations of hot spot regions in MYH7 genes in Iranian hypertrophic cardiomyopathy patients

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Hypertrophic cardiomyopathy occurs approximately 1 in 500 people. It is the most common cause of sudden cardiac death in young people. The disease is characterized by hypertrophy of left and/or right ventricles and intraventricular septum. Mutations in at least 11 genes (such as MYH7, MYBPC3 and TNNT2) encoding sarcomeric proteins and possibly in one gene encoding a non-sarcomeric protein (PRKG2) have been associated with HCM so far. 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. 52 Iranian patients with hypertrophic cardiomyopathy were selected. Exons 13-15 and 19-21 of MYH7 gene and their related introns were amplified by polymerase chain reaction. Then PCR products were sequenced. Mutations were detected in fourteen (28%) of the patients. Three mutations were found in exons. We have found a novel mutation, A10419C in exon 14, which was a missense mutation causing N444T substitution. Mutations in the MYH7 gene can be found in patients without a family history of HCM

P0069. Mucopolysaccharidosis II: Biochemical and Linkage Study of the Iduronate-2-sulfatase Gene Defects in Taiwan

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Mucopolysaccharidosis II (MPS II) is an X-linked recessive lysosomal storage disorder caused by a defect of the iduronate-2-sulfatase (IDS) gene. To investigate the molecular lesions underlying Taiwanese MPS II, 14 probands and families were identified and screened for IDS mutations by DNA sequencing and restriction analysis. Three novel (IVS2+1G>C, 1055del12, and G489D) and 7 previously reported (N63K, P228L, K347E, R468Q, R468W, I485R, and 1241delAG) mutations were found. R468Q and R468W together account for 42.8% of mutations detected in our patients. Haplotype analysis using flanking probes DDX1113 and DDX1123 revealed that the unrelated R468Q alleles are independent in origin whereas the unrelated R468W alleles are probably of the same origin. The R468Q mutation in one patient and the I485R in another patient occurred de novo in male meiosis. Leukocyte IDS assaying showed significantly different ranges of activities in normals and the carriers (19.2~70.6 vs 8.4~26.6 nmol/h/mg cell protein). The mean IDS activity in female carriers was less than a half of the normal level. However, due to a small overlapping range, the level of enzyme activity alone can not be used for carrier detection.

P0070. Report of Cockayne Syndrome from Iranian Families

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Cocayne Syndrome is an autosomal recessive multisystemic condition, characterized by usually senile-like changes beginning in infancy.

Retinal degeneration, impaired hearing and photosensitivity of thin skin.

The disease is known to be genetically heterogeneous for which 3 different Loci have been identified on chromosomes 10, 12, 13.

We have studied five Iranian families, each with one affected child (three male, two female). Three of these cases are results of consanguineous marriage and the parents of the two cases are offspring of unrelated couples. Their features and radiology were compatible with Cockayne Syndrome.

The ophthalmologist showed salt&pepper retinal pigmentation for two of the affected children and optic atrophy for other three cases.

Assays of DNA repair are performed on skin fibroblasts. The most consistent finding in Cockayne Syndrome, fibroblasts, marked sensitivity to UV radiation, deficient recovery or RNA synthesis following UV damage (and impaired repair of) activity transcribed genes, or transcription couple repair.

PND of CS has been reported by analysis of UV light sensitivity and DNA repair in fetal cells obtained by CV or Amniocentesis.

P0071. Chromosomal disorders in patients with azoospermia

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The aim of this study was to analyse chromosomal alterations in patients with azoospermia, candidates for testicular sperm retrieval (TESE) and intracytoplasmatic sperm injection (ICSI). The last three years 66 biopsies were performed in 64 patients for TESE. Mean age of the patients was 35 years (range 24-53).

According to estimations, in 15% of infertile males it is a genetic disorder that stands in the background of seminal weakness. Male infertility can be traced back to numerical or structural chromosomal causes in 5% of the cases. Approximately 70% of these causes are due to sexual chromosome abnormalities, the most frequent being the XXY syndrome. Microdeletion of the Y chromosome is around 10%.

In our study the most characteristic cases were numerical deviations, such as XXY, XYY and mosaic XO syndromes. In order to obtain an exact diagnosis, the traditional cytogenetic methods (Q-, G-band and FISH analysis with probes X painting, Y painting, X cen, Y heterochromatin, Y euchromatin) were complemented in combination with molecular genetic techniques (AzFa, AzFb, AzFc region). Patients were included in the assisted reproduction programme on the base of their genetic results.

Non obstructive azoospermia was diagnosed in 51 patients (79%). Spermatozoa adequate for ICSI were found in 36 cases (54%), fertilisation rate was 57% and pregnancy rate 27%. Preoperative evaluation included routine andrological investigation with 2 semen analysis, ultrasound, hormonal and genetic examination. Cryopreservation of retrieved testicular tissue was also done.

P0072. Genetic and cellular analysis of Italian patients with a Nijmegen breakage-like phenotype

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Normal development of the immune system requires the introduction of DNA double strand breaks (DSBs) during antigen receptor gene assembly; defect in the repair of these damage can lead to profound immunodeficiency.

The Mre11/Rad50/Nbs1 (M/R/N) complex plays a critical role in the repair of DSBs; in humans, mutations in the *Nbs1* gene lead to the

autosomal recessive genetic disorder Nijmegen breakage syndrome (NBS).

Lymphoblastoid cells (LCLs) derived from five Italian NBS-like patients selected in relation to their clinical aspects were studied from a cellular and molecular point of view to determine defects in factors involved in DSBs repair.

PCR amplification of exon 6 showed the presence of both copies of the *Nbs1* gene in all the investigated individuals. Normal levels of proteins involved in the repair of DSBs and in cellular response to DNA damage (NBS1, Mre11, Rad50, etc.) were detected.

Radiosensitivity of some of the LCLs analysed was shown by the induction of chromosome aberrations in a G2-phase assay. Rejoin of DSBs was evaluated by PFGE.

LCLs have been irradiated with 2-10 Gy of X-rays and proteins subjected to immunoblot with a p53 antibody, NBS1 and SMC1. Histone γ-H2AX focus formation after irradiation was also evaluated. Dissection of the clinical and cellular phenotype of NBS-like patients will help to identify a subset of individuals with the NBS clinical phenotype, whose analysis will allow for more clear-cut research of new genes involved in the maintenance of genome integrity.

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P0073. Elejalde Syndrome, a Case Report & Branchio-Oculo-Facial Syndrome, a Case Report

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Acrocephalopolysyndactylous dysplasia, Elejalde syndrome (MIM 200995) and branchio-oculo-facial syndrome (BOF, MIM 113620) are rare genetic disorders.

Elejalde syndrome includes macrosomia, craniosynostosis, facial anomalies, short limbs, lung hypoplasia, organomegaly, excess subcutaneous tissue of neck and trunk. Excessive amounts of connective tissue and perivascular proliferation of nerve fibres is found in many organs. The mode of inheritance is autosomal recessive, the appropriate gene has not been identified yet.

We present a patient with Elejalde syndrome born at 36 weeks of gestation. The infant died 11 hours after birth. This is the 7th described case of Elejalde syndrome.

Branchio-oculo-facial syndrome includes somatic changes, such as bilateral postauricular haemangiomatic skin defects, low set ears, preauricular fistulas, cleft lip or cleft lip and palate, nasolacrimal duct obstruction, eye changes, malformed nose, hearing loss, growth retardation and renal dysplasia, aplasia or agenesis. Intelligence is mostly normal. Inheritance appears to be autosomal dominant, sporadic cases probably carry new mutations, however, the corresponding gene has not been identified so far. The situation is complicated by the clinical overlap with branchio-oto-renal syndrome (BOR, MIM 113650). This observation led to a suggestion that BOF and BOR are allelic variants of the same gene. However, new studies have shown, that they are distinct entities.

A girl with a full manifestation of branchio-oculo-facial syndrome is presented.

P0074. Evolutive possibilities of Crigler-Najjar syndrome type I in two siblings

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Introduction: Crigler-Najjar type I syndrome is a very rare genetic disease with autosomal recessive inheritance. The disease begins in the first days of life with the occurrence of an intense jaundice, with values of indirect serum bilirubin higher than 20 mg/dl, in the absence of hemolytic manifestations or liver function alteration. The cause is the absence of hepatic UGT_{1A1} (uridine diphosphate glucuronyl transferase) activity. Early diagnosis in Crigler-Najjar type I syndrome is very important for preventing kernicterus in newborn.

Methods: We followed two siblings having Crigler-Najjar type I syndrome who were admitted in our service. The first one (R.G., male, now 12 years of age) was admitted in our service right after birth with intense jaundice. The suspicion of a Crigler-Najjar syndrome or an hemolytic jaundice was raised. The second one (R.F., female, now 10 years of age), the fourth child of the family, was admitted at the age of

3 months with intense jaundice, spastic palsy, opisthotonus. Diagnosis of Crigler-Najjar type I syndrome was almost certain because the kernicterus was present and she had a brother with this disease.

Results: R.G.: bilirubin (total) = 22mg/dl; bilirubin (indirect) = 21.9mg/dl; Hb=15g/dl; phenobarbital stimulation did not influence the serum level of bilirubin. Genetic tests showed a defect of 2q3.7. R.F.: bilirubin (total) = 20mg/dl; bilirubin (indirect) = 20mg/dl; Hb=14.9g/dl

Conclusion: Because in the second case no therapeutical measures were taken (phototherapy) kernicterus occurred. Molecular tests can prove the severity of the syndrome. Genetic advice is important for future births.

P0075. HLA DRB1*04 alleles in autoimmune hepatitis patients-frequence and relationship with response to treatment

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Autoimmune hepatitis (AIH) is a rare frequent, multiplex disorder with undefined etiology. HLA DRB1*04 has been identified as independent determinant of susceptibility to AIH, and HLA DRB1*04 positives are more likely to develop extrahepatic manifestation of their disease. We analyzed the association of HLA DR4 in Iranian patients with AIH. METHODS: HLA DRB1*04 genotyping was done for 70 patients (47 female) with AIH type I (defined by international criteria IAHG, 1999 and fulfilled informed consent) referred to Taleghani hospital (Iran) and 95 healthy blood donors (61 female) matched by sex and age, respectively. PCR-SSP (PCR-Sequence Specific Primers) was used to detect HLA DRB1*04 allele. RESULTS: Mean age \pm SD and the results of evaluation of AST (Aspartate aminotransferase) and ALT (Alanine aminotransferase) are shown in table 1. The frequency of HLA DRB1*04 allele are shown in table 2. There was no significant difference in HLA DR4 allele frequency between cases and controls ($p>0.05$). HLA DRB1*04 positive AIH patients had higher AST and ALT. CONCLUSION: Our results do not confirm the association between HLA DR4 allele and autoimmune hepatitis in Iranian population but there is association between HLA DR4 allele and response to treatment in Iranian patients with autoimmune hepatitis.

Table 1		
	Controls	Cases
Mean age \pm SD	35.4 \pm 13.4	35.3 \pm 11.5
AST	22.85 \pm 10	381 \pm 593
ALT	29.53 \pm 16	347 \pm 573

Table 2						
	Cases	Cases	Controls	Controls		
	Male	Female		Male	Female	Total
HLA DRB1*04 positive	4	14	18	8	18	26
HLA DRB1*04 negative	19	33	52	26	43	69

P0076. Fragile X premutation Tremor/Ataxia Syndrome (FXTAS): an Italian Collaborative Study

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FXTAS has recently been described in carriers of the premutation in the FMR1 gene. The clinical presentation includes gait ataxia or intention tremor at onset, associated with a wide spectrum of neurological symptoms. A first study showed that the prevalence is age-dependent, ranging up to 75% in subjects older than 80 years (Jacquemont 2004). No data are available about the relationship between the number of

CGG repeats and the age at onset or the clinical presentation.

Based on a network of geneticists, neurologists and neuroradiologists, a concerted effort was initiated in order to recruit a large sample of male subjects from extended families in which the presence of FMR1 premutation was ascertained, and to investigate the occurrence of FXTAS. Premutation carriers, as well as a matched series of control individuals, will be examined for the presence of movement disorders and other neurological disturbances, through an extensive protocol which includes neuroimaging. Number of repeats, FMR1 mRNA and FMRP levels will be evaluated and their possible correlation with clinical features will be tested. Furthermore, the knock-in mouse model will be used to investigate the effects of the premutation at the cellular and molecular levels. We have already identified, in 266 families, 93 premutation carriers and 239 possible carriers. A telephone survey was carried out in a subgroup of families, in collaboration with the Italian patients' association: 19 out of 51 male premutation carriers (37%) were referred to as showing symptoms suggestive of FXTAS.

P0077. Presentation of one case with Larsen syndrome recessive

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This syndrome is characterized by joint hypermobility, multiple joint dislocation, characteristic facies (Prominent forehead, depressed nasal bridge, wide-spaced eyes, Club foot, bilateral dislocation of elbows, hips and knee (most characteristically, Anterior dislocation of the tibia on the femur). Mutant gene is located on 3p14-3p21. Our case is a 4.5 years old boy from first cousin parents that he had bilateral dislocation of elbows and hip, prominent forehead, depressed nasal bridge, scoliosis, short and hypoplastic carps and joints laxity. Two children of his uncle with consanguineous parents are suffering from similar signs and symptoms. Radiologic studies for the parents of affected children were normal.

P0078. Extremely severe microlissencephaly associated with microgenitalism: a new syndrome distinct from XLAG?

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We report on a non consanguineous family with a severe form of microlissencephaly associated with microgenitalism, consistent with an X-linked pattern of inheritance. There are two affected sib ships of five males, born of two different fathers and sharing the same mother. Prenatal diagnosis of severe brain malformation and underdeveloped genitalia was made on ultrasound in all cases. Four pregnancies were terminated and pathological examination was performed in three foetuses, reporting identical anomalies in each of them. Extremely severe microlissencephaly was associated with absent corpus callosum, but cerebellar development was normal. Hemispheres were completely sideways divided with opened ventricular cavities in the cranium and excess of cephalo-rachidian fluid. Major disturbance of early cortical organization and neuronal migration with nodular heterotopias of the white matter was noted. One twin pregnancy was followed since only one foetus was affected. The affected child lived until the age of 4 and presented with neonatal onset intractable epilepsy, poor temperature regulation and chronic diarrhoea.

To our knowledge, this particularly severe type of X-linked microlissencephaly associated with microgenitalism has never been reported in the literature and could be linked with mutations in an unknown gene involved in early cortical organization. Although the cerebral disorder in these cases differs in some respect with the X-linked lissencephaly with ambiguous genitalia (XLAG), molecular analysis of ARX gene was performed. The results are still pending.

P0079. Intraparietal sulcus anatomical abnormalities in children with Williams syndrome

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Williams syndrome (WS) is a well defined syndrome on both clinical and molecular levels. Indeed, owing to the size of the hemideletion of the WS critical region in 7q11.23, the syndrome encompasses more or

less of clinical features among supravalvular aortic stenosis, severe infantile hypercalcemia, growth retardation, dysmorphic features and neurobehavioral disabilities. While language and auditory short-term memory seem to be relatively spared, visuo-spatial constructive cognition disabilities are a constant hallmark of the WSCP. In order to search for putative structural abnormalities underlying such a specific neurodevelopmental disorder, we performed an anatomical magnetic resonance imaging (MRI) in 9 children (12 +/ 2.5 years) using optimized voxel-based morphometry (VBM). VBM is a fully automated whole-brain technique that delivers a voxel-wise assessment of regional grey and white matter concentration. The control group was composed by normal age-matched children. A significant bilateral decrease of grey matter concentration was detected in the intraparietal sulcus of WS children ($P < 0.05$ corrected height threshold). The location of the present abnormalities coincides with the location of the structural abnormality with the same method in 13 WS adults. These intra-parietal abnormalities are consistent with cognitive profile of WS; the dorsal stream dysfunction is probably involved in the visuospatial construction deficit of WS patients. A better neuroimaging characterisation in WS should contribute to better define phenotype-genotype correlations in this syndrome.

P0080. Molecular screening of C6orf57 as a candidate gene in autosomal recessive retinitis pigmentosa linked to RP25 locus.

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RP25 locus was identified as a novel locus for autosomal recessive retinitis pigmentosa (arRP) responsible for a considerable (14%) proportion of the arRP families of Southern Spain. It lies in the pericentromeric region of chromosome 6, between D6S257 and D6S1644 microsatellite markers. Here we report the mutation screening of the chromosome 6 open reading frame 57 gene (C6orf57) in 7 families of the South of Spain linked to RP25 locus.

C6orf57 was selected according to its location within RP25 region and its tissue expression, since it appears to be highly expressed in peripheral retina. A molecular genetic study was performed on DNA extracted from one parent and one affected member of each studied family. The genomic structure was determined using bioinformatic tools. The molecular analysis revealed four single nucleotide polymorphisms (SNPs) and a 3'-UTR AT- repeat polymorphism (IVS1-38T>C, IVS2-122G>A, Q46R, STOP+106A>G and (AT)n). The genotyping of 3'-UTR AT- repeat polymorphism in all the members of the affected families linked to RP25 and in 100 Spanish controls showed that the highly repetitive and homozygous alleles are significantly over represented in the RP patients. The well-known role of the 3'-UTR AU-rich elements in the stability of mRNA makes it plausible the hypothesis that C6orf57 could be implicated in the etiology or severity of RP in some of the RP25 families. Therefore, further functional studies are required for the assessment of the influence of this AT-repeat polymorphism on C6orf57 mRNA.

P0081. Mutation screening of three candidate genes, TFAP2β, GLULD1 and RIM1, in autosomal recessive retinitis pigmentosa.

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Retinitis pigmentosa (RP) is the most common form of retinal dystrophy. It is featured by a great clinical and genetic heterogeneity. There can be found different patterns of inheritance, such as autosomal dominant and recessive, X-linked and digenic, exceeding 36 the number of identified RP loci. Here we report the identification and exclusion of 3 candidate genes for autosomal recessive RP (arRP), the most frequently inherited form of RP, in 7 Spanish families with arRP linked to the pericentromeric region of chromosome 6. TFAP2β, GLULD1 and RIM1 were selected on the basis of their location, function and tissue expression. TFAP2β, transcription factor AP-2 beta, is selectively expressed during early differentiation of amacrine and horizontal cells. GLULD1, glutamate-ammonia ligase (glutamine synthase) domain

containing 1, plays a key role in the uptake of glutamate in retina. RIM1, encoding a presynaptic protein involved in the glutamate neurotransmission, constituted as well a good functional candidate for a RP causing gene. Two polymorphisms were identified in the GLULD1 gene, one of them changed the encoded amino acid (F246L) while the other one did not result in any substitution (E430E). The third change, silent as well (L222L), was found in RIM1. Genotyping of the SNPs was undertaken using Fluorescence Resonance Energy Transfer (FRET). These changes, however, were also present in Spanish controls. Although TFAP2β, GLULD1 and RIM1 were excluded as the causative genes for these RP families, we could not rule them out as good candidates for other retinal degenerations mapping to the same chromosomal region.

P0082. Trisomy 10 mosaicism in 2-years-old normally developed girl

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Total trisomy 10 has been detected in spontaneous abortions and stillborn. In the literature there were only four reported live born cases with trisomy 10, all of them mosaic. Although the trisomic clone have not exceed 30% in none of the cases all have demonstrated prenatal growth retardation and early death before the age of six months. Here we report on a 2-month-old female followed until the age of 2 years with total trisomy 10 mosaicism. Pathological vs. normal karyotype has been found in peripheral lymphocytes and in cultured fibroblasts, respectively in 54% and 22%. Interphase FISH confirmed these cytogenetical results finding respectively 51% and 26% trisomic cells. The affected girl shows physical and mental development in the normal range. Her dysmorphic features include left-sided cleft lip and median cleft palate (found prenatally by US screening), high prominent forehead, delayed closure of the fontanelles (35/35mm at the age of 11 months), epicanthus, hypertelorism, delayed teeth eruption, both-sided single palmar crease, deep palmar and plantar creases. No inner anomalies have been found. Our case is the longest survival of a total trisomy 10 mosaicism reported so far and the only one with normal development, although it demonstrates comparable cytogenetical data with other literature reports.

P0083. Association of multiple supernumerary teeth with cleidocranial dysplasia

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Dental anomalies, including failure of eruption of permanent dentition or supernumerary teeth, are the major diagnosis criteria in cleidocranial dysplasia (CCD). Two cases with multiple supernumerary teeth as part of a genetic syndrome are presented. **Objective:** the aim of the study was to investigate the phenotype-genotype correlation. **Subjects and Methods:** the two patients with supernumerary teeth in association with CCD were investigated. The diagnosis of dental anomalies has been made by oral examination and evaluation of orthopantomographs. Physical and X-ray examinations had a valuable role in supporting clinical diagnosis of CCD. Family study, cytogenetic and molecular analyses were performed too. **Results:** Case 1: a 16-year-old female expressed failure of exfoliation of the primary dentition and failure of eruption of permanent teeth. She also presented the classical signs of CCD - complete absence of the clavicles and frontal bossing. Her family history shows no other affected members. Because CCD is usually inherited as an autosomal dominant disorder and about one-third of the cases appear to represent sporadic mutation, we suspected a new mutation has arisen. The results of cytogenetic and molecular analyses validate the occurrence of a new mutation in exon 2 of RUNX2 gene. Case 2: a 14-years-old female showed major problems in the eruption of permanent teeth. The girl presented partial absence of the clavicles. Her family history revealed an autosomal dominant pattern of inheritance. **Conclusions:** the two cases of CCD presented variability in clinical features and etiology. The phenotype cannot be predicted based on the genotype.

P0084. Intracranial hypertension in two children with Marfan syndrome

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Two unrelated children are presented with recurrent intracranial hypertension. One child was known to have Marfan syndrome. In the other child the diagnosis Marfan syndrome was made during evaluation for the intracranial hypertension.

Both children presented with complaints of headache, nausea and vomiting and one of them had papilledema. Both had increased cerebrospinal fluid (CSF) pressure (50-80 cmH₂O) and the complaints disappeared after a lumbar puncture. One child was treated with a ventricular peritoneal drain because of the frequent relapses of intracranial hypertension. The other child has episodes of intracranial hypertension with intervals of about a year and is treated with lumbar punctures so far.

Although severe headache has been reported in Marfan syndrome due to intracranial hypotension, this is to our knowledge the first report of intracranial hypertension in Marfan patients. Our hypothesis is that our patients may have reduced dural resistance as a consequence of decreased plasticity of collagen due to the fibrillin defect leading to cerebral venous compression impairing the cerebrospinal fluid resorption.

P0085. Multiple basal-cell carcinomata of the skin, jaw keratocysts, skeletal and other malformations

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Basal cell carcinoma syndrome (BCNS) has been described by Gorlin and Goltz in 1960 as an autosomal dominant condition. The main symptoms are multiple basal-cell carcinomata of the skin and jaw keratocysts and skeletal malformations.

We report here about 8 families with BCNS. The condition is located at 9q233 and shows high degree of penetrance. A number of loci and possible candidate regions were in the past excluded.

50 % our patients had the initial symptomatology in the orofacial area. Recurrent jaw keratocysts are the major symptoms - 50% patients). The pits - small palmar or plantar dyskeratoses, in the previously part of the suffered are present. Constant the numerous skeletal anomalies clinically and/or radiographic examinations were observed.

We mentioned increased incidence of malignancies. In one case we noted ovarian carcinomas and the second patient had meningioma. The benign conditions we registered - in one case the cutaneous fibromas of the trunk and in one female myomatosis uteri.

Family members were classified as affected if they exhibited at least two of following criteria by the age of 18: two ore more basal cell carcinomata,odontogenic keratocysts, palmar pits or skeletal malformations. We noted unusual case of primary hyperparathyreoidism in a women with nevoid basal cell carcinoma syndrome. The adenoma of parathyreoid gland has not yet been described in patients with BCNS.

All patients from our 8 families had macrocrania, wide bicristal diameter and small distantia biacromialis, 6 from 8 families indicated autosomal genetic transmission, 2 are presented as fresh mutations or gonadal mosaicism.

P0087. Array CGH detection of genomic imbalances in mentally retarded individuals with normal G banded karyotypes.

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Mental retardation affects about 3% of the general population. Causative relationship for the underlying genetic alterations could be established in less than half of the cases. In order improve the diagnosis we have used array comparative genomic hybridization to detect genomic imbalances in cases mental retardation with dysmorphic features and normal karyotypes after G banding.

In 22 of the 82 tested patients (27%) we have detected genomic imbalances from which 7 were de novo, 10 were inherited and 5 cases the origin could not be determined.

A major limitation for the use array-CGH in genetic counselling is the identification of those inherited imbalances that contributes to the abnormal phenotype.

Although the introduction of array-CGH as a diagnostic tool for clinical genetics seems expensive and technically demanding, the resulting efficiency favours its implementation: for each 100 arrays performed, genetic counselling, and eventually pre-natal diagnosis, of 12-28 families will be improved.

P0088. Severe neonatal hypertrophic cardiomyopathy caused by compound heterozygous mutations in the MYBPC3 gene

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease characterised by unexplained left ventricular hypertrophy and by a characteristic histopathological appearance, myofibrillar disarray. The disease is mainly caused by mutations in genes encoding sarcomeric proteins. One of the most frequently mutated HCM genes is the myosin binding protein C (MYBPC3) gene, (MIM 115197). Mutations in this gene generally cause a relatively mild phenotype with a late age of onset. HCM in neonates is rare and often associated to another underlying condition.

A full-term girl was born after an uneventful pregnancy and delivery. She was the second child of healthy non-consanguineous parents. On the third day of her life she was admitted because of poor drinking, cyanosis and difficult breathing. Further investigation showed pulmonary oedema and cardiomegaly. ECG registration was abnormal. Echocardiography revealed hypertrophic non-obstructive cardiomyopathy with a poor left and right ventricular systolic function. There was no structural congenital heart defect present. The heart failure was progressive leading to death at the age of 5 weeks. Autopsy confirmed marked hypertrophy of both ventricles, myofibrillar disarray and interstitial fibrosis were demonstrated by electron microscopy. DNA-studies showed two mutations in the MYBPC3-gene: maternally inherited mutation c.2373_2374 insG and paternally inherited splice-donor mutation c.1624+1G>A. Further cardiologic examination confirmed HCM in the father. Mutation c.2373_2374 insG is a founder mutation originating from the Netherlands.

These findings suggest the need for mutation analysis of genes encoding sarcomeric proteins in childhood HCM and the possibility of compound heterozygosity.

P0089. Identification of NBS1 gene mutation carriers by PCR

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Nijmegen Breakage Syndrome (NBS) is an autosomal recessive disorder characterised by microcephaly, immunodeficiency, hypersensitivity to X-irradiation and increased predisposition to lymphoid malignancies.

The product of the underlying gene, NBS1, is a 95 kDa protein called nibrin, a member of the hMRE11/hRad50 protein complex, involved in the repair of DNA double-strand breaks.

Over 90% of all NBS patients are of Central and Eastern European origin and are homozygous for the 657del5 mutation in exon 6. Nine further mutations have been found in families of different ethnic origin. These rarer mutations are located between nucleotides 657 and 1142 and are also predicted to truncate the nibrin protein downstream of the N-terminal domain, as observed for the classical 657del5.

A PCR method to rapidly detect the private mutations 742insGG and 835del4 in exon 7 and 900del25 mutation in exon 9 of NBS1 gene was developed. In particular, NBS1 specific primers for wild-type and mutated alleles were designed and for each mutation a specific PCR protocol was optimized. This method was applied to analyse three unrelated NBS1 families, two from Italy and one from Morocco.

The rapid and inexpensive screening technique, that we introduced in this study, could be used for larger population screening in order to estimate NBS heterozygotes frequency. Since NBS heterozygotes

might be expected to have increased risk of cancer and high sensitivity to irradiation, the identification of carriers of mutant NBS1 alleles will results helpful in cancer therapy, possibly optimising treatment protocols for malignant disease.

P0090. Ehlers Danlos syndrome type VI in a girl

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Ehlers Danlos syndrome (EDS) type VI is a rare autosomal recessive connective tissue disease, involving primarily skin and joints. The main features of the condition are neonatal hypotonia, scoliosis and as rare complications, ruptures of arteries and the eye globe. We present a 4 year old girl born to consanguineous parents with multiple skeletal anomalies at birth: torticollis, bilateral dislocation of shoulders and hips, scoliosis and bilateral talipes equinovarus. At the age of 4, she presented with thin, bruiseable, hyperelastic and easily stretchable skin with multiple petechiae, visible subcutaneous vasculature, without scarring, omphalocele and significant hypotonia, joint laxity and scoliosis. Intelligence was normal for her age. The ratio of urinary lysyl / hydroxylysyl pyridinolines was increased. Sequencing of the *PLOD1* gene revealed a homozygous deletion in exon 13 (c.1362delC) leading to a frameshift and premature truncation of the lysyl hydroxylase (p.Ile454IlefsX2) and thus confirmed the clinical diagnosis of EDS type VI. As our patient was diagnosed at a young age, a therapeutic trial with ascorbic acid 2gr/kg was initiated 1 year ago. No major complications from the cardiovascular system or the eyes have been noted so far. In this family, prenatal testing was performed and a normal fetus was predicted.

P0091. Mutation screening for atypical and adult onset Spinal Muscular Atrophy

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The Spinal Muscular Atrophies (SMAs) are a group of genetically determined conditions characterised by the congenital or progressive loss of lower motor neurons with the subsequent weakness and atrophy of skeletal muscles. The majority of cases present in childhood and are due to recessive mutations affecting the gene *SMN1*. A less common presentation involving slowly progressive SMA, often predominantly affecting distal muscle groups and presenting for the first time in adulthood is also well described. In this study we investigated the role in the aetiology of adult onset SMA of mutations in 5 recently described genes; Glycyl tRNA synthetase (*GARS*), the small heat shock proteins *HSPB1* and *HSPB8*, the Berardinelli-Seip congenital lipodystrophy gene (*BSCL2*) and the vesicle trafficking protein *VAPB*. The role of these genes was investigated in 74 individuals affected by SMA with a variety of clinical phenotypes. A pathogenic mutation was detected in each of the *GARS*, *HSP27*, *HSP22* and *BSCL2* genes. In the first 3 families the affected individuals had a pure motor neuropathy with lower motor neuron involvement only. In the family affected by a *BSCL2* mutation a mixture of upper and lower motor neuron signs were present, consistent with the previously described Silver Syndrome phenotype. Sequence variants were detected in a further 10 families. The mutations found in this group demonstrate that while the *GARS*, *HSP27*, *HSP22* and *BSCL2* genes each make a contribution to the incidence of adult onset SMA it remains a genetically heterogeneous condition with further contributing genes yet to be discovered.

P0092. Effectiveness of the diagnosis of fragile X syndrome in mentally retarded patients from the West Siberia of Russia

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A comparison of the results both cytogenetic and molecular diagnosis of the syndrome Martin-Bell (SMB) in mentally retarded patients from

the Western Siberia in Russia mainly young boys, was performed. Two groups of patients were examined: a group of 205 unrelated patients with mental retardation and minimal clinical features of SMB and 21 patients with Prader-Willi syndrome-like phenotype of SMB without muscular hypotonia and abnormality of chromosome 15. Molecular diagnosis included PCR analysis of the length CGG-repeats and assay of the methylation status CpG islands of the gene *FMR1*. The fragile site on distal part of the long arm chromosome X was detected in 14.6% cases. It was shown that the disease frequency in these patients confirmed by molecular assays was only 66.7%. On the other hand, the frequency of SMB in males with mental retardation without fragile X-chromosome was 2.8%. We have found, that the minimal level of fragile site expression for correct diagnosis SMB is 4%. On the whole, the frequency of the SMB in patients with mental retardation, long face, hyperactivity, emotional disinhibition and joint hypermobility in patients from the Western Siberia in Russia was 10.2%. The value of this index in patients with Prader-Willi syndrome-like phenotype of SMB without muscular hypotonia was 4.8%.

P0093. The molar tooth sign phenotypes: evaluation of clinical features in 65 patients

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The Molar Tooth Sign (MTS) is a radiological finding that reflects a complex midbrain and hindbrain malformation. Although the MTS is the cardinal diagnostic sign in Joubert syndrome (JS), it has been also found in several other conditions named Joubert Syndrome Related Disorders (JSRD) in which the JS phenotype is associated with the variable involvement of other organs, mainly the eye, kidney and liver. The clinical classification of JSRD is still complex, as incomplete and overlapping phenotypes exist and the complete spectrum of associated features still needs to be elucidated. We recruited 65 patients with proven MTS and evaluated their features. All patients presented psychomotor delay associated with hypotonia/ataxia (92%), mental retardation (87%), oculomotor apraxia (86%), breathing abnormalities (40%) and epilepsy (15%). Up to 25% of cases presented additional central nervous system malformations, including hydrocephalus and corpus callosum anomalies (11% each), encephalocele (6%), neuronal migration defects and Dandy-Walker/variant malformation (4% each). Overall, 67% of patients showed ocular involvement, mostly a retinopathy (46%), optical nerve atrophy (15%), ocular motility abnormalities (14%) and colobomas (9%). Kidney involvement was observed in 35% of patients, of which 27% had nephronophthisis/urinary concentration defects (evaluated with DDAVP test) and 6% had cystic dysplastic kidneys. Liver abnormalities and polydactyly were found in 17% and 15% respectively. Rarer associated features included congenital heart defects, cleft palate, intraoral frenula or lingual hamartomas, pituitary hamartomas and Hirschsprung disease. The characterisation of the MTS associated features is relevant to delineate a diagnostic algorithm to timely manage multisystemic complications of JSRD.

P0094. The Frequency and Structure of Congenital Malformations in Infants of Rostov Region Rostov-on-Don Scientific Research Institute Of Obstetrics and Pediatrics. Ministry of Public Health of Russian Federation

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Monitoring of congenital malformations (CM) in infants of Rostov Region started in 2000 as a part of a State Public Health Programme. It provided 3,256 medical infant which became available thanks to the data of various Public Health Institutions of Rostov Region (Maternity Hospitals, Children hospitals and Out-patients clinics, Prosecution service). The total number of newborns in Rostov Region comprised 110,741 for the period mentioned, while the total number of infants with congenital malformations was 1,614.

The frequency of the occurrence of CM in infants of Rostov Region comprised 14,5 %.

All congenital malformations were subdivided into several groups according to the systems affected, as follows: 1. cardiovascular system abnormalities - 22,06%, 2. central nervous system abnormalities - 11,76%, 3. bone and muscular system abnormalities - 10,76%, 4.genitourinary malformations - 9,23%.

Basic frequencies of CM determined by monitoring were as follows: Unencephalia - 0,21%; spinabifida 0,60%; encephalocele - 0,07%; congenital hydrocephaly - 0,54%; microtia, anotia - 0,09%; cleft palate - 0,35%; cleft lip and/or cleft palate - 0,82%; transposition of the great vessels - 0,017%; hypoplasia of left heart - 0,06%; esophagoatresia - 0,18%; anal atresia - 0,08%; renal agenesis or renal dysgenesis - 0,14%; hypospadiasis - 1,23%; exstrophy of the bladder - 0,02%; reduction malformations of extremities - 0,19%; congenital diaphragmatic hernia - 0,14%; omphalocele - 0,14%; gastroschisis - 0,26%; Down's syndrome - 1,40%; multiple congenital malformations 2,15%.

The monitoring results obtained show that the spreading of certain nosologic forms of CM in a population of Rostov Region is similar to that in other populations.

P0095. An unstable intermediate allele in a family with the Fragile X syndrome

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A 1 year and 4 months old toddler presented with a developmental delay. He showed mildly dysmorphic features with length and head circumference above p80. A maternal cousin had a developmental delay that had never been analysed. Investigation of the FMR-gene as part of the work-up for mental retardation showed a full mutation as found in persons with classic Fragile X syndrome. Further analysis in this family showed that female carriers of the syndrome showed so-called intermediate alleles in the FMR-gene. In this family, a normal/intermediate allele became a full mutation in two generations. A review of the literature is given regarding the significance of the size of premutations and their risk to be transmitted to next generations as full blown mutations.

P0096. Focal facial dermal dysplasia : congenital, bilateral vesicular facial lesions with a linear and symmetric distribution.

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We present three unrelated children with distinct congenital facial skin lesions.

Patient 1 had a unilateral right cleft lip and cleft palate with late closure of the left lip. She presented congenital symmetrical skin lesions in an arc across each cheek starting preauricularly. These appeared as vesicles, with absent underlying skin, and contained a limited amount of fluid. Follow-up revealed normal development. Patient 2 sustained an unexplained large left-sided intracerebral haemorrhage perinatally and developed seizures. Two symmetric, vesicular lesions, ½ to 1 cm in diameter were noted on each cheek at birth. One new lesion, more

medially placed, erupted on each side at about 2-3 weeks. Patient 3 had a small chin and somewhat cupped ears, 1 nevus on the left foot. There were symmetrical lesions on both cheeks at birth which have persisted and have a collar of lanugo-like hair. This condition has been reported in the dermatological literature as focal facial dermal hypoplasia with *preauricular* localisation. Familial occurrence, compatible with dominant as well as recessive inheritance have been reported. The pathogenesis probably involves fusion defects of the mandibular and maxillary prominences of the developing embryonic face, which suggests that the cleft lip and palate seen in patient 1 are not coincidental.

P0097. 22q11.2 deletion in a series of paediatric at risk patients

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Background: 22q11.2 deletion syndrome is a common disorder typically consisting of dysmorphic facies, congenital heart defects (CHD), hypoparathyroidism, immunodeficiency and palate abnormalities. The associated phenotypic manifestations are extensive, highly variable from patient to patient and age-dependent. Patients with mild clinical manifestations presenting with apparently isolated malformation or dysmorphic traits can be easily overlooked. **Objecitve:** to find out if the 22q11.2 deletion studies should become a part of a standardized diagnostic workup for patients presenting with isolated defects or with dysmorphic traits alone. **Methods and patients:** We prospectively studied the frequency of 22q11.2 deletion in an unselected population of 171 patients aged 4 days to 18 years referred because of: 1. CHD (64) 2. cleft palate (58) 3. hypocalcemia (18) 4. dysmorphic features suggestive of del22q11.2 (31). Detailed clinical evaluation, high-resolution chromosome and FISH analysis were performed. **Results:** FISH analysis revealed 22q11.2 deletion in 9.4% (6/64) patients with CHD. In the subgroup of patients with conotruncal anomalies del22q11.2 was present in 17.8% (5/28) patients. From 18 patients referred because of the hypocalcemia, 6 had 22q11.2 deletion (33,3%). In the group of 31 patients with dysmorphic features, the diagnosis was confirmed in two patients (6.4%). **Conclusions:** Testing for the 22q11.2 microdeletion can be recommended in all patients with conotruncal heart defects and in patients with hypocalcemia. It could be considered in patients presenting with at least 3 dysmorphic traits suggestive of 22q11.2 microdeletion syndrome. A routine screening for the 22q11 deletion patients with an isolated palatal defect may not be warranted.

P0098. Familial amyotrophic lateral sclerosis. Genetic and Clinical studies of eighteen cases in a Spanish family

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting motor neurons. Up to 20% of the patients have a family history of ALS (FALS), most commonly inherited as an autosomal dominant trait. Mutations in the SOD1 gene have been identified in about 20% of FALS individuals.

We have identified a large Spanish family with 18 cases of FALS in six generations. In three of the cases, the coding region (exons 1-5) of the SOD1 gene was amplified with PCR and sequencing was carried out using ABI Prism 377 DNA sequencer. The identified mutation, affecting the 21st codon in exon 1 (Glu21Gly), is already known in ALSorg database, although with few clinical data, and it is the sixth mutation of the SOD1 gene described in Spain.

The mean age of onset was 47.2 years old, and the mean time of duration was 16.11 years, with a range 5-21. Symptoms in most of the cases (88,25%) started in the lower extremities and the remaining in the upper extremities. Neither bulbar symptoms nor dementia were observed. Electromyogram demonstrated electrophysiologic evidence

of lower motor neuron involvement in the control case (in which mutation was positive).

In conclusion, the Glu21Gly mutation showed long time of evolution with intrafamilial variability and homogeneous phenotype. The existence of two unaffected obligate carrier demonstrate that penetrance could be incomplete.

P0099. Amelogenesis Imperfecta (AI) and Nephrocalcinosis : a rare or unrecognized syndrome ?

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Amelogenesis Imperfecta (AI) is a clinically and genetically heterogeneous group of disorders of enamel development. Enamel defects can occur in isolated or syndromic forms. Mutations in genes encoding for enamel matrix proteins (*Amelogenin X*, *Enamelin*) have been identified respectively in X-linked and autosomal dominant families with apparently non syndromic AI.

The AI-Nephrocalcinosis (AIN) or renal-enamel syndrome is an autosomal recessive condition with only few reported cases in the literature. Renal symptoms include progressive nephrocalcinosis without any apparent abnormality in calcium metabolism that could lead some patients to renal failure in the adulthood. Mutations of *Kallikrein 4*(*KLK4*), a gene encoding for an enamel matrix proteinase have been recently identified in an autosomal recessive AI family. The kallikrein-kinin system has been implicated in glomerulotubular development and *KLK4* could be a good candidate gene for this syndrome. We report two consanguineous families and three sporadic cases of AIN. Generalized enamel hypoplasia of both primary and permanent dentition was present in all affected patients. In two families delayed or absent molar eruption and microdontia were noted. None of the patients had impaired renal function. In one sibship, nephrocalcinosis was only present in 1/3 patients. As nephrological screening is not systematically done in AI patients, the incidence of this syndrome could be underestimated, and the risk of renal failure overestimated. As early diagnosis may lead to a better renal prognosis, all children with AI should at least have a renal ultrasonography performed to exclude nephrocalcinosis.

P0100. New cardiac findings in a case of Kabuki Syndrome

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Kabuki syndrome is a rare multipl congenital anomaly/mental retardation syndrome with an estimated frequency of 1/32.000 in Japan. The Kabuki syndrome characterized by mental retardation (mild to moderate), skeletal abnormalities, postnatal growth deficiency, typical facial appearance and congenital heart defects. The incidence of congenital heart defects in patients with kabuki syndrome is estimated to be about 30%. To date, no specific type of heart malformation is known to be associated with the syndrome.

We report a girl Who shows postnatal growth deficiency, a mild microcephaly with moderate mental retardation and skeletal abnormalities (hip dislocation, pectus excavatum, scoliosis, end plate abnormalities of vertabral body) and dysmorphic facies (arched eyebrows, long palpebral fissures, large and protruding ears) and congenital cardiac defects. Cardiac echo revealed perforation of mitral valve, mitral insufficiency and anomalous pulmonary venous return. We propose to investigate these cardiac findings Kabuki syndrome patients survey in attempt to determine their real frequency and in order to improve clinical management.

P0101. Lack of association between Leber's hereditary optic neuropathy primary point mutations and multiple sclerosis in the region of Calabria (Italy).

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The hypothesis that mitochondrial genes may implicate susceptibility to multiple sclerosis (MS) is supported by an increasing number of case reports on Leber's hereditary optic neuropathy (LHON) - associated mitochondrial DNA (mtDNA) point mutations in patients with MS. A number of mtDNA mutations with primary pathogenic significance for LHON, a maternally inherited disease causing severe bilateral visual loss predominantly in young men, have been detected in patients with an MS-like phenotype. To evaluate the link between MS and LHON primary point mutations, we investigated a cohort of non-related clinically definite MS Calabrian patients with optic nerve involvement, as well as a cohort of patients without involvement of the optic nerve as controls. Each subject recruited to the study was fully informed and gave his/her consent. We searched for the presence of LHON mitochondrial mutations at nucleotide positions (np) 11778, 3460 and 14484 by mutation-specific polymerase chain reaction and restriction fragment length polymorphism. Our results suggest that there is no association between Calabrian patients with typical MS and mtDNA point mutations at np 11778, 3460 and 14484. However, the presence of further mitochondrial mutations cannot be excluded in MS.

P0102. Novel CACNA1A gene mutations in Spanish patients with episodic ataxia, familial hemiplegic migraine and infantile paroxysmal vertigo.

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Mutations in the CACNA1A gene, encoding the $\alpha 1A$ subunit of the P/Q-type calcium neuronal channel, have been associated with the allelic disorders familial hemiplegic migraine (FHM), episodic ataxia type 2 (EA-2) and spinocerebellar ataxia type 6 (SCA6).

We have performed extensive mutational analysis of the CACNA1A gene in 41 unrelated patients with one or more of the following diagnoses: FHM, migraine with hemiparesis aura (MA), a childhood periodic syndrome, EA-2 and progressive ataxia (PA).

For each proband, the whole coding region of the gene and the exon-intron boundaries were PCR-amplified from genomic DNA and sequenced, allowing the identification of five new and one previously reported missense mutations in 6 patients.

Two of the novel mutations, p.G638D and p.P1011A, were found in EA-2 patients. The novel mutation p.A454T was detected in a patient with MA and panic attacks. The other two new mutations were associated with FHM; p.V581M was carried by a pure FHM patient and p.Y1245C by a patient with FHM and antecedent of paroxysmal torticollis. The previously described p.R583Q mutation was present in a patient with PA and FHM and was found to co-segregate with EA-2/FHM/PA in 5 other affected relatives. No mutations were found in the CACNA1A gene in the remaining 35 probands, confirming genetic heterogeneity in this group of channelopathies.

The clinical spectrum of CACNA1A mutations should be expanded to include some childhood periodic syndromes. Further clinical, genetic and functional studies are needed to elucidate the molecular bases of this group of related paroxysmal disorders.

P0103. Frequency of the GAG deletion of the DYT1 gene in a group of Polish patients with primary dystonia

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Dystonia is a heterogenous movement disorder characterized by sustained muscle contractions, frequently causing twisting movements or abnormal postures. To date, fifteen well-defined types of inherited dystonia syndromes or primary torsion dystonias (PTD) are known. Most cases of early-onset primary generalized dystonia cases are caused by a 3-base-pair GAG deletion in *DYT1* gene (9q34).

The aim of our study is to investigate the prevalence of the GAG deletion in the *DYT1* gene in Polish patients with primary dystonia. The preliminary analysis was carried out in a group of 50 patients (aged 11-68 yrs) with primary dystonia (28 with generalized type; 22 with focal/segmental of various localization), unreactive to L-Dopa treatment.

Detailed clinical characteristics of all the patients is provided. Four patients (8%) in the analyzed group carried the GAG deletion in the *DYT1* gene. They all had generalized-type dystonia starting in the lower limb, with age of onset ranging from 7 to 14 yrs. Progression of symptoms followed the pattern: foot → entire lower limb → trunk → upper limb/s.

In patients' families, additional 7 carriers of that deletion, of whom 5 were asymptomatic, were identified. Generalized, early-onset symptoms were observed in one of symptomatic carriers. In the other, writer's cramp was the only manifestation.

Our data confirms that: generalized phenotype, early onset, primary foot involvement and presence of one additional affected member in the family can be predictors of type 1 dystonia. Recognition of *DYT1*-positive patients allows consideration of deep brain stimulation (DBS) as a method of effective treatment

P0104. Clinical and molecular studies in 15 patients with Noonan syndrome

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Noonan syndrome (NS, OMIM 163950) is an autosomal dominant genetic disorder characterized by following clinical symptoms: short stature, congenital heart defects, pectus deformities, pterygium colli, mild mental retardation and facial dysmorphisms: hypertelorism, epicanthic folds, low-set, prominent ears. Recently discovered *PTPN11* mutations (gene located on 12 chromosome p24.1) are responsible for NS in almost half the cases. Results of preliminary clinical and molecular studies carried out in selected group of 15 patients with clinical diagnosis of NS are reported. The following inclusion criteria for molecular testing were used: dysmorphic features, short stature, congenital heart defects, pectus deformities or pterygium colli and cryptorchidism. Detailed clinical evaluation, including family history, dysmorphic features, pre- and postnatal development and congenital malformations, was performed.

The most frequent symptoms in our group of patients were: dysmorphic features (hypertelorism (100%), low-set ears (79%), short neck (93%)), congenital heart defects (93%), short stature (79%) and pectus malformations (57%).

To determine the character of mutation in *PTPN11* gene, we performed a direct sequencing analysis of the exons 2-15. Mutation in *PTPN11* gene was identified in 4 of 15 investigated patients. One mutation c.846C>G in exon 7 is novel; two others were previously reported - c.188A>G (exon 3) and c.1510A>G (mutation inherited from mother). All patients with *PTPN11* mutation demonstrated typical symptoms of NS, such as: short stature, congenital heart defects, wide spaced nipples, pterygium colli and thick ear helix. The pulmonary stenosis (typical for NS) was found only in one patient, whereas the other three had different type of cardiac malformations.

P0105. Intrauterine and postnatal growth retardation due to a familial heterozygous missense mutation in the IGF-I receptor

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Intra-uterine and postnatal growth retardation with elevated plasma IGF1 has been reported in 2 children with mutations in the IGF1 receptor (IGF1R) gene. We now report a mother and daughter with growth failure due to a novel heterozygous IGF1R mutation.

Mother had a birth weight of 2.6 kg (-1.9 SDS) and a length of 49 cm (-0.34 SDS). She had unexplained growth failure. GH provocation tests at 6 and 12 years showed elevated responses. At present (34 yrs), height is 144.6 cm (-4.0 SDS) and head circumference is 50.2 cm (-3.0 SDS). IGF1, IGF2 and IGFBP3 are all in the high-normal range.

After a pregnancy complicated by oligohydramnion the daughter was born with weight 2.1 kg (-3.3 SDS), length 42 cm (-4.2 SDS), OFC (at 2 months) 33.3 cm (-5.6 SDS). She has a triangular face, mild hypotelorism, a small mouth with thin lips and prominent ears. She had poor appetite, and at 13 months of age length was 69.2 cm (-2.6 SDS),

weight 6.3 kg (-3.0 SDS), head circumference 39.7 cm (-6.1 SDS). Plasma IGF1, IGF2 and IGFBP3 were normal. An arginine stimulation test showed an elevated response (max GH 67 mU/L).

A heterozygous missense mutation in exon 16 of the IGF1R gene (G3193A) was found, changing a negatively charged glutamic acid for a positively charged lysine. We conclude that heterozygosity for an inactivating mutation in the IGF1R leads to a variable degree of intrauterine and postnatal growth retardation, suggesting that maternal IGF1R is involved in intrauterine growth.

P0106. Three children with structural chromosomal aberrations and clinical features of overgrowth syndromes

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Chromosome abnormalities are reported in a substantial part (4% - 28%) of the individuals with mental retardation. They are the single most common known cause found in series of unselected patients with mental retardation. Some of the chromosomal disorders usually have highly recognizable physical characteristics. In other cases the abnormality may be presented by small rearrangement of a particular chromosome that is rarely reported and the phenotype is nonspecific or still undetermined. Overgrowth syndromes include infants who are large for gestational age with or without excessive postnatal growth. The definition also includes increased weight, length, or head circumference, and/or asymmetric enlargement, singly or in combination.

We present three unrelated mentally retarded children with structural chromosomal abnormalities and clinical features suggestive of overgrowth syndromes. Patient 1 is a 2 years old girl with karyotype 46,XX,der(10)add(10)(q26)

where the FISH analysis showed that the additional material probably originates from chromosome 10. Patient 2 is a 16 months old girl with 22q13 deletion, detected on conventional karyotyping and confirmed by FISH analysis. Patient 3 is a 14 years old boy with karyotype 46,XY, add(5)(q35) where the additional material was shown to originate from the distal part of the 10p, i.e. this patient has partial trisomy 10p. In all patients the chromosomal abnormalities were de novo.

These cases confirm the necessity of chromosomal analysis in patients with mental retardation and somatic overgrowth.

P0107. DLL3-mutations in spondylocostal dysostosis type1 (SCDO1)- report of 2 patients

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The spondylocostal dysostosis (SCD) are a heterogeneous group of disorders with severe axial skeletal malformations, characterised radiologically by multiple vertebral segmentation defects and rib anomalies, mainly reduction in number and dorsal fusion.

An autosomal recessive form, designated SCD type1 (OMIM #277300) is linked to 19q13, which harbours the DLL3-gene (delta like 3). DLL3 encodes a ligand for the Notch signalling pathway.

Clinically the patients present with an extremely short neck, low set nuchal hairline, a short chest, abdominal protrusion and an increased abdominal pressure. Therefore, inguinal hernia occurs frequently in male patients. Interestingly, despite of severe vertebral malformations, neurological complications are uncommon. Scoliosis is fixed and not progressive. The two male patients were born to consanguineous Turkish parents.

Patient 1: born at term, c-section, BW 3380g, L 48cm, HC 36cm, no respiratory problems at birth, mild symptoms, slightly disproportionate stature. Homozygous missense mutation in the DLL3-gene C209R within the delta-serrate-lag2 region (DSL)

Patient 2: born at term, BW 2960g, L 43 cm, HC 34cm, developed a respiratory distress syndrome and was ventilated for 24h hours. Reduced compliance of the chest was noticed. An right sided inguinal hernia was operated in the neonatal period. Homozygous insertion of cDNA 603_604 ins GCGGT in exon 5 of DLL3 leading to a stop codon and protein truncation .

We stress, that an essential prerequisite for genetic testing of DLL3 is that there is irregular formation of **all** vertebrae (not segmental) usually

in association with abnormally aligned ribs, showing points of fusion.

P0108. Investigation of mtDNA Deletions and D-Loop Polymorphism in Iranian hypertrophic cardiomyopathy patients

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Hypertrophic cardiomyopathy (HCM) is a genetic disorder. It has variable presentations with a high incidence of sudden death in young. The disease occurrence is %0.05-0.2. As mitochondria are the major sites of energy production in the cell, it is not surprising, therefore, that an energy dependant tissue such as heart is affected by mitochondrial dysfunction. The aims of this study were investigating mitochondrial DNA deletions, identifying polymorphic sites in D-loop region of mtDNA and potential genetic background accounting for HCM in the Iranian population. In this study 52 unrelated HCM patients underwent genetic test on blood sample by DNA Extraction, PCR, and complete length sequencing of mtDNA. D-loop was sequenced for 31 patients. The presence of mtDNA deletions was analyzed by multiplex PCR method and deletions break point were confirmed by sequencing. The sequences were aligned upon the Cambridge Reference Sequence. Common deletion in 18 patients (15.3%), a 7.3 kb deletion in 7 patients (13.4%) and a 9kb deletion in 12 patients (23%) were detected. Deletions were localized to an area including genes for COX I, II, III, ATPase, ND3 to ND6, and cyt b. In D-loop, the majority of mutations were nucleotide substitutions. Transitions (91%) were significantly higher than transversions (8.3%). Thirty four polymorphisms were newly identified in this study, not 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.

P0109. Case report of Usher syndrome in mother and daughter

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Usher syndrome is a clinically and genetically heterogeneous disorder characterized by congenital hearing loss and later development of progressive retinitis pigmentosa. It is one of the most frequent causes of deafness-blindness in humans. The present report is the case of a Caucasian mother and her daughter both born with Usher syndrome. Both had hearing impairment since birth and onset of visual impairment due to pigmentary retinopathy in late teens. **Objective:** to describe the clinical manifestation of Usher syndrome in this family and to study the phenotype-genotype correlation. **Methods:** Tests as visual acuity, refraction, biomicroscopy, ophthalmoscopy, visual field, electroretinography and audiometry were helpful in diagnosis of Usher syndrome. **Results:** both mother and daughter were diagnosed with Usher syndrome type Ila with different clinical manifestation (sensorineural hearing loss and visual acuity varied in severity; neurological abnormalities and posterior subluxicular cataract as a later complications of the mother); family history revealed maternal greatgrandmother suffered from Usher syndrome and a familial predisposition for open-angle glaucoma; the family pedigree suggested an autosomal recessive mode of transmission for the disorder; no consanguineous marriage was noticed ; cytogenetic analysis showed no abnormalities in 1q41. **Conclusion:** because of inevitable blindness as part of Usher syndrome phenotype the children who inherited the mutant gene from their parents should be tested for early diagnosis. Electroretinogram testing is recommended in children with bilateral sensorineural hearing loss.

P0110. A terminal deletion of the short arm of chromosome 3 [46, XY, del(3)(p25-pter)]: Report of a Case

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Hemizygous deletion of the short arm of chromosome 3: [46 XY,del(3)(p25-pter)] was reported to be associated with failure to thrive, microcephaly, characteristic craniofacial anomalies, mental retardation and musculoskeletal anomalies. Only 35 cases with a small deletion of the distal segment of 3p have been reported. Differences in the

deletion size have been noticed to be associated with variability in the clinical manifestations. We report a 13-year-old patient with severe mental retardation, dysmorphic facial features, seizures and other characteristic findings of 3p deletion phenotype. This is the first reported patient reported with deletion of the von Hippel-Lindau (VHL) gene, included in the deletion at the 3p25 segment. Von Hippel-Lindau gene deletion is known to be associated with a risk of developing the VHL disease including cerebellar hemangioblastoma, renal cell carcinoma and retinal angioma. These features have not been reported yet as part of the 3p telomeric deletion syndrome. The clinical manifestations, genetic counselling and findings on follow up are reported.

P0111. Hereditary multiple exostoses disease- clinical and genetic aspects

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Hereditary multiple exostoses (HME) is characterized by growth of multiple exostoses, which are benign cartilage-capped bone tumors that grow outward from the metaphyses of long bones. HME has a prevalence of 1 in 100 000 and is inherited in an autosomal dominant manner, with 95% penetrance. We report 15 cases of HME over a 20 years period. The diagnosis was established by clinical findings and comprehensive skeletal radiographs in individuals with exostoses arising in the juxtaphyseal region of long bones, exostoses grow in size and gradually ossify during skeletal development and bowing of the forearm or lower leg as associated deformity. The median age at diagnosis was 2 years and a half. 2 cases associated multiple exostoses with a cardiac malformation and with talipes respectively. The number of exostoses, number and location of involved bones, and degree of deformity varied. Most commonly involved bones were the tibia, the femur and the humerus. Males tended to be much frequent affected and more severely affected than females. The family history appeared to be negative in 40% of affected individuals, and we supposed a new gene mutation. Solitary exostosis have been found in 4 female cases. Symptoms arised secondary to mass effect were pain, motor deficits, mechanical blocks to motion. The height of affected individuals failed within the normal range. We found no sarcomatous degeneration of an exostosis. None patients with hereditary multiple exostoses needed surgery. We could not study the causal genes EXT1, EXT2 and EXT3 and we consider the cases open for molecular studies.

P0112. Two siblings with postaxial polydactyly, a congenital heart defect, ectopic pituitary gland and distinct facial features. A new entity?

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We present two female siblings with a previously unreported syndrome. The parents are unrelated and family history is negative with regard to congenital malformations or mental handicap. Both siblings presented with unexplained intrauterine growth retardation (birth weight respectively 2640g (at term) and 1200 g (at a gestational age of 32 weeks)). Both had bilateral postaxial polydactyly of the hands, a congenital heart defect (a perimembranous ventricular septal defect (VSD) in the first child, a VSD, pulmonary stenosis and an atrial septal defect secundum type A in the second child). Brain MRI revealed the presence of an ectopic neurohypophysis gland in both children and additionally a hypoplastic adenohypophysis gland in the youngest sibling. Developmental delay was severe in the first child, the second child died at the age of 4 months. Both children had a marked facial dysmorphism, sparse coarse hair and temporal balding. Metabolic testing was normal. High resolution karyotype was normal. To the best of our knowledge, this constellation of features has never been described before. Autosomal recessive inheritance is likely.

P0113. Short stature of prenatal onset in a girl with terminal deletion 15q including the *IGF1R*-locus

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We report on a girl that was referred to our department because of short stature. She is the first child of a healthy, non consanguineous German couple. The pregnancy was complicated by growth retardation, first recognized during 25th week of gestation. Normal birth took place in the 37th week with reduced birth measurements: weight 1630g (-3.6 SD), length 37 cm (-4 SD), OFC 30.5 cm (-2.8 SD). After birth, a heart defect (ASD) and clubfeet were diagnosed. Because of an occipital swelling with overlying haemangioma, an MRI scan was performed that showed an atric cephalocele. X-rays revealed a hemivertebra of the lumbar spine and fused ribs 1 and 2 on the right. Bone age was retarded by one year at the age of 18 months.

Clinical examinations at the age of 1 and 3 years showed microcephaly (-4.2 SD/-3.5 SD), short stature (-5.6 SD/-5.6 SD), dystrophy, facial dysmorphic signs (high forehead, short nose, small mouth) and clinodactyly of fifth fingers. Developmental delay was only mild, except for walking with 25 months (2 months after correction of clubfeet).

Cytogenetic investigations showed normal results, as well as UPD analyses for chromosomes 7, 14, 16, and 20. Subtelomeric screening showed a subtelomeric deletion 15q which occurred de novo (normal results in the parents). Further investigations to define the breakpoint revealed that the *IGF1R*-locus is also deleted.

In the literature, most patients with terminal deletions 15q have a more severe phenotype with mental retardation, but patients with ring 15 show similarity with our patient.

P0114. Phenotype/Genotype in 349 patients with Noonan syndrome (NS): ongoing French collaborative study

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Introduction

NS represents a multiple congenital anomaly entity characterised by dysmorphism (triangular face, hypertelorism, ptosis, downslanting palpebral fissures, thick helix, low set, posteriorly rotated ears; deep philtrum, pterygium colli), cardiac defect, short stature, cryptorchidism in male, mental retardation. Mutation in *PTPN11* gene was identified in ~40% of patients

Objective

Delineate phenotype/genotype correlation in a large cohort and provide clinical tools to optimize genotype screening

Methods

Geneticist (France, Belgium, Switzerland) recruited patient with clinical diagnosis of NS and for each, completed a booklet with antenatal, perinatal, growth parameters, cardiopathy, dysmorphic features, dermatologic anomaly, psychomotor milestones, pictures over time. Patients were included if they presented dysmorphic features and one additional anomaly. Informed consent, DNA sample and booklet were sent at Hôpital Robert Debré - reference center for NS in France. Patients with LEOPARD, Costello, CFC, Baraitser-Winter syndrome were excluded for this study. Mutation analysis performed by bi-directional direct sequencing on exons 2,3,4,7,8,12 and 13 on *PTPN11* gene (> 98% of reported mutation)

Results

349 patients were included. The mutation rate was 36% (126/349): 41.15% males (79/192) and 29.90% females (47/157). Among the studied parameters (see Methods), there were not statistically different between mutated and non mutated groups but for a positive correlation for cardiac defect: pulmonic stenosis 53.2% vs 26.5% -(p<0.001) and atrial defect: 25.4% vs 11.2% -(p<0.001). Inability to identify a mutation was associated with presence of hypertrophic cardiomyopathy.

Delayed mental development is noted in 34% of the cohort (30% in the mutated group vs 36% in the non-mutated group)

P0115. Hyperphosphatasia with cognitive deficit and seizures: further syndrome delineation

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Hyperphosphatasia is a persistent elevation of serum alkaline phosphatase activity seen in many disorders, particularly those affecting bone turnover. There have been occasional reports of hyperphosphatasia associated with progressive postnatal neurodevelopmental disease and seizures (MIM#239300). Affected children exhibit consistently elevated alkaline phosphatase (ALPL) of uncertain origin. There are no other laboratory findings of note and no evidence of any abnormality of bone or liver metabolism, or altered skeletal architecture. Affected siblings and/or consanguinity suggest autosomal recessive inheritance, but the underlying pathogenesis is unknown.

We describe a new case and review the clinical features in the context of 16 previously reported children. Our proband is a 5½ yr-old girl born to consanguineous parents, who presented with marked developmental delay and dysmorphic features (brachycephaly, coarse facies, hypertelorism, bulbous nose with prominent nasal bridge, and downturned corners of the mouth) at five months of age. Subsequently, failure to thrive, acquired microcephaly, and autistic behaviours were observed. Seizures and an abnormal EEG were also seen. The hyperphosphatasia (867±72 IU/L, n=7) is associated with decreased pyridoxal 5'-phosphate (B₆), and the question of aberrant vitamin B₆ metabolism has been raised in this condition before. Pyridoxine challenge (100 mg bolus) resulted in normalized EEG activity. The patient's tonic-clonic and absence seizures have not been evident since phenobarbital withdrawal and treatment with 100 mg pyridoxine daily. Our case illustrates two points: 1) alkaline phosphatase should be assayed in children presenting with similar findings; 2) pyridoxine-EEG challenge should be attempted to determine whether other affected children show pyridoxine-responsiveness.

P0116. Rare coexistence of Saethre-Chotzen phenotype with 46,X,add(X),(p21) karyotype

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Classic Saethre-Chotzen syndrome (SCS, acrocephalosyndactyly type III, MIM#101400) is characterized by coronal synostosis, facial asymmetry, ptosis, and characteristic appearance of the ear. Mutations in the TWIST1 gene, chromosomal locus 7p21, are associated with this syndrome. Occasionally, affected individuals have chromosome translocations involving 7p21 or ring chromosome 7. Some patients with an overlapping phenotype have a mutation in the FGFR3 gene (4p16.3) and in the FGFR2 gene (10q26). Our proband, one-and-a-half-year-old female, with SCS phenotype is under our observation. She was born after the third pregnancy to a 34-yr-old female and 40-yr-old male by spontaneous vaginal delivery. Proband parents are not consanguineous. Her mother is healthy, her father, father's mother have mild hearing loss. Proband birth weight was 2100g (25th centile), length was 49cm (< 3rd centile). She had some distinctive features that were typical for CSC: at the age of one-and-a-half year her length was 76cm (5th centile); her weight was 8.2kg (<3rd centile); acrocephaly, open fontanelle, flat face, high forehead, mild facial asymmetry, maxillary hypoplasia, shallow orbits, hypertelorism, ptosis, beaked nose, narrow palate, broad chest, mild syndactyly of the 2nd-3rd fingers, syndactyly of the 3rd-4th toes, hallux valgus, sacral sinus. Moderate sensorineuronal deafness was revealed with otologic examination. Proband's karyotype was found to be 46,X,add(X),(p21). Having used routine chromosome analysis we had no chance to identify the character of this additional piece of X chromosome. Her parents karyotype was normal.

P0117. Phenotypic characterization of two patients with Rett syndrome carrying mutations in exon 1 of the human MECP2 gene

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Rett syndrome (RTT) is a neurodevelopmental disorder inherited in an X-linked dominant manner. The clinical course is characterized by near-normal development during the first months, followed by a decline of acquired functions, progressive microcephaly and severe developmental delay. The gene responsible for RTT, MECP2, functions as a general repressor of transcription. About 70-80% of RTT cases are attributed to de novo mutations detected by sequence analysis, in 16% of patients with a classical RTT phenotype large deletions of MECP2 are present.

The human MECP2 gene comprises 4 exons whereof the first exon has been considered as non-coding. Very recently, however, an alternative splice variant composed of exons 1, 3 and 4 has been identified, utilizing exon 1 as protein-coding sequence. This new isoform appears to be the predominant MECP2 transcript in the brain.

So far, no details have been reported on the clinical picture of patients with mutations of the new MECP2 isoform. We have therefore screened patients with suspected RTT in whom sequencing of exons 2, 3 and 4 did not reveal MECP2 mutations for the presence of large deletions and point mutations in exon 1 by DNA sequencing and multiplex ligation-dependent probe amplification (MLPA). We identified one girl with a large deletion and one girl with a nonsense mutation in exon 1, most likely affecting the new MECP2 isoform. A detailed clinical description of these patients will be given.

P0118. Deletion of chromosome 13 in a child with bilateral retinoblastoma, dysmorphic features and developmental delay due to a maternal insertional translocation

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Retinoblastoma (RB) is the most common pediatric intraocular tumor with an incidence of 1/20,000 liveborns. The majority of cases are sporadic. RB is caused by a loss or inactivation of both alleles of the RB1 gene located at 13q14.1-14.2. In 5% of cases RB is the result of a constitutional chromosomal deletion involving 13q14. There have been five families (15 patients; 10 patients from one family) reported in the literature where individuals with RB had 13q14 deletions resulting from malsegregation of a familial insertional translocation involving 13q14 and various other chromosomes.

We report a 21 month old dysmorphic female with bilateral RB, congenital heart disease and developmental delay. The family history was positive for a maternal uncle who had RB and died at age two years. Chromosome analysis of the proband revealed a 13q14 deletion [46,XX,del(13)(q14.11q21.2)] due to the malsegregation of a maternal insertional translocation [46,XX,ins(8;13)(q23.3;q14.1q2 1.2)]. The child's mother was 11 weeks pregnant at diagnosis. CVS revealed a fetus with the same 13q14 deletion as seen in the proband. A literature review of previously described cases of familial insertional translocations with deletions of 13q14 and RB reported developmental delay in all cases and dysmorphic features and/or congenital anomalies in 13 of 15. Cytogenetic analysis is indicated in all cases of RB as 5% have a chromosomal deletion or structural rearrangement. A karyotype is necessary for precise genetic and prenatal counseling.

P0119. Somatic microsatellite mutations in spontaneous abortuses

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In the mutation rate analysis of 10 microsatellite tetranucleotide DNA repeats in 95 spontaneous abortuses with normal karyotype and 51 control group of medical abortuses, 4 embryos (4,2%) demonstrating

presence of additional alleles absent in both parents were found. In the group of medical abortuses we observed no mutational events for investigated loci what allow to exclude hypermutability of these markers as a reason of increased mutation rates in the spontaneous abortuses sample. The difference between two groups is statistically significant ($p < 0.05$). Appearance of the "new" alleles which is absent in both parents serve as consequence of the somatic mutation occurred during embryogenesis. In spontaneous abortuses, the mean mutation rate in the tetranucleotide repeat complexes analyzed was $0.886 \cdot 10^{-2}$. This value was higher than the mean spontaneous mutation rate of these human STR loci. It can be suggested that genome instability detected at the level of repeated DNA sequences can involve not only genetically neutral loci but also active genomic regions crucial for embryonic viability. This results in cell death and termination of embryonic development. Our findings indicate that the death of embryos with normal karyotypes in most cases is associated with an increased frequency of somatic microsatellite mutations.

P0120. Does Xp22.3 deletion cause mental retardation? The contribution of VCX-A and NLGN-4 to cognitive development

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Patients with Xp22.3 interstitial and terminal deletions have been shown to be affected by mental retardation or autism. Previously, VCX-A (variably charged protein X-A), located at Xp22.3, was introduced as a gene for mental retardation and its presence was suggested to be sufficient to maintain normal mental development. Recent reports suggest that mutations in NLGN4 (neuroligin 4), located at that same region, are involved in autistic disorders and mental retardation.

In the current study we describe a pedigree of 3 generations affected by contiguous gene syndrome that includes the clinical features of X-linked ichthyosis and Kallmann syndrome. Molecular analysis revealed the presence of an interstitial deletion spanning approximately 3.5Mb at Xp22.3. The centromeric breakpoint was localized between markers DDX1467 and DDX8051, proximal to KAL-1. The telomeric breakpoint was localized within the coding region of NLGN4. The deletion of VCX-A and the truncation of NLGN4 in this family prompted us to examine the cognitive functions of our two adult patients using comprehensive intellectual and neurocognitive assessment. Normal intellectual function was found in one patient and only mild mental retardation was revealed in the other. Neither patient met any DSM-IV criteria for a pervasive developmental disorder such as autism. These results suggest that normal mental development can be achieved despite the deletion of VCX-A and the partial deletion of NLGN4, emphasizing the importance of environmental factors and suggesting the possible contribution of modifying genes.

P0121. Cytogenetic profile on cataract patients in and around Coimbatore city

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An experiment was conducted on 50 Cataract patients with different morphological categories. Among the total cataract patients, only 7 patients exhibited chromosomal abnormalities, in that 3 of senile cataract patients, 2 of Posterior sub capsular, 1 of Hyper mature and Anterior subcapsular. In the Senile cataract type 2 Female grouped under group I and II and a male grouped under I exhibited 46 XX or XY, (5;10) (p 11;q23) of a translocation between chromosome 5 and 10. The similar kind was detected in a female grouped under I with anterior subcapsular cataract. In the hyper mature type a single male displayed 46 XY, t (4; 9) a translocation between chromosome 4 and 9. Two cases of male observed with a translocation of chromosome 5 and 10 (46 XY, t (5; 10) (p11; q24) in posterior subcapsular cataract. All the chromosomal abnormalities formed in patients were having O+ blood group (except one experimental male A+ blood group). In the present study showed that there is a close relationship between cataract and group "O" being recognized.

P0122. Interleukin-1 β and its receptor antagonist gene polymorphism and the risk of respiratory distress syndrome in neonates from Russia

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Respiratory distress syndrome (RDS) develops in an infant within the first few hours after birth and manifests as respiratory failure and deficient gas exchange. It is caused by a deficiency of pulmonary surfactant due to immaturity of the lung. There are a number of potential interactions between surfactant and cytokine effects on the preterm lung. Cytokines may be regulators of surfactant metabolism in the preterm infant. So genes that encode cytokines would be plausible candidate genes for RDS, such as interleukin-1 (IL-1) or receptors antagonist interleukin-1 (IL-1RN).

Biallelic C/T polymorphisms at positions IL-1 β -511 and IL-1 β +3953 and 86-bp VNTR polymorphism in intron 2 of IL-1RN have been described. These polymorphisms are located within the regulatory regions of the genes and are of potential functional importance by modulating IL-1 protein production.

Objective of this pilot study are to determine whether these polymorphisms is related to RDS. We determined genotypes and alleles frequencies of IL-1 β and IL-1RN genes in infants with RDS from Russia (n=76) and healthy control group of neonates (n=36) using PCR. The obtained results showed, that the distribution of CT genotype of IL-1 β (C-511T polymorphism) among RDS group and control group was 51,6% versus 38,8% ($\chi^2=1,01$, $p=0,31$, OR=1,68). The frequencies of other genotypes, alleles and haplotypes in RDS group were not differing from control group.

We concluded that polymorphic variants of IL-1 β gene may play role in pathogenesis of RDS. There are no association between C+3953T polymorphic variants of IL-1 β and IL-1RN genes and RDS predisposition.

P0123. Familial Cases of Androgen Insensitivity Syndrome (AIS)

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30 patients 16 and 27 years of age with the diagnosis androgen insensitivity syndrome (AIS) have been studied in the Zhordania institute of Human Reproduction for 10 years. There were 3 familial AIS cases, where two sibs were affected with AIS. All patients have undergone chromosomal, clinical, ultrasound and hormonal examination, pedigrees of families have been studied as well. In two families both sibs have complete androgen insensitivity syndrome (CAIS) and in the third family one patient has CAIS and the other-partial androgen insensitivity syndrome (PAIS). In all cases karyotype was 46,XY. Phenotype includes, normally developed breast, scanty or absent pubic and axillary hair, blind ending vagina, normal male levels of testosterone. In the history of patients inguinal hernias and existence of testes in the hernial sac was frequent in 16 patients (53,3%). In the third family intrafamilial genotypic and phenotypic variation was observed. 21 years old one sib with CAIS had gonads located in abdomen. 20 years old sib had PAIS, mild hirsutism, one testicle located in pubic area and at the age of 13 with the diagnoses of abdominal hernia unilateral gonadectomy was made. This case shows, that in the same family there can be observed different types of AIS.

P0124. Saethre-Chotzen Syndrome due to a novel TWIST mutation: phenotypic variability in a three generation family

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We report on a German family in which Saethre-Chotzen syndrome (SCS) was diagnosed after the birth of a girl with highly asymmetric frontal bone, very wide fontanelles, hypertelorism, asymmetric orbits and flat mid face. X-ray showed asymmetry of the skull basis and the orbits, an anterior fontanel reaching up to the root of the nose and asymmetry of the mandibula. Neurosurgical correction was performed at the age of 2 years. At present the girl is 5 years old and shows normal development. Retrospectively, SCS was also found in the father and his mother who both have hypertelorism without a marked facial asymmetry. Both also report on a delayed closure of unusually wide

fontanelles. The grandmother additionally has got cutaneous syndactyly of the 4th and 5th toes, a feature often associated with this syndrome. Meanwhile, a second child was born also showing mild signs of SCS. The phenotypic variability in our family is in accordance to the literature underlining the necessity for a careful clinical examination of the parents as diagnostic clue. Molecular analysis of the TWIST gene revealed a mutation (409 A>G) not previously described which leads to an amino acid exchange in position 137 (T137A). The mutation is located in the functional bHLH-domain, a highly conserved sequence area, where a clustering of missense mutations associated with SCS is observed. Therefore, it is very likely that the mutation leads to a loss of function of the protein and can be regarded as disease causing.

P0125. Prenatal diagnosis of femoral-facial syndrome

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We describe a case of femoral-facial syndrome (FFS) detected prenatally on second-trimester sonography. Fetal abnormalities shown by sonography included microgenia, bilateral hypoplastic femurs and bilateral talipes. The diagnosis was confirmed after birth. FFS is a rare sporadic syndrome with femoral hypoplasia and unusual facies. The facial features include upplanting palpebral fissures, short nose with broad tip, long philtrum, thin upper lip, microgenia, and cleft palate. The femora are mostly bilaterally affected and they are short with lateral bowing. Upper limb involvement is possible. In one third of cases the mother has diabetes mellitus. Mental development in FFS is normal. Stature is short due to short legs. There are therapeutic options for microgenia and short femurs. If microgenia and short, bowed femora are found on prenatal sonography FFS should be suspected.

P0126. Osteocalcin Gene Hind III Polymorphism and bone mineral density in children with insulin-dependent diabetes mellitus

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Insulin-dependent diabetic mellitus (IDDM) individuals are known to develop disorders of bone metabolism resulting in osteopenia. The osteocalcin gene (OG) allelic variant HH was found to be overrepresented in women with osteopenia. The purpose of the present study is to determine the relationship between the OG polymorphism and BMD in children with IDDM.

120 IDDM children (62 girls, 58 boys) were examined. The mean patient was 12.1 \pm 3.7 years. Testing was done on all patients to determine the Hind III polymorphisms in the promotor region and serum levels of intact osteocalcin, calcitonin, Ca^{2+} , phosphate, parathyroid hormone, 25-OH-vitamin D levels, haemoglobin A(Ic), insulin measurement as well as radiographs and forearm osteodensitometry.

Using the data of BMC we selected the children in two groups (with and without osteopenia). The distribution in the group with (without) osteopenia was: HH 4.5% (6.7%), Hh 25.4% (33.3%) and hh 70.1% (60%). There were no significant differences between OG polymorphism and BMD in those groups. However, when IDDM children were controlled for sex and BMD we found some statistical significance ($\chi^2 = 4.5$, $P=0.1$).

No significant correlation was found between plasma osteocalcin levels and osteocalcin polymorphism.

In summary, our preliminary study shows some relationship between the polymorphism of the osteocalcin gene, sex and BMD in children with IDDM, further research on this issue is needed.

P0127. Familial complex 10p;12p rearrangement unravelled by subtelomeric FISH analysis

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Subtelomeric analysis has been performed in over 2,500 MR individuals, with a 6% yield. Therefore, it is indicated as a second tier test after HRB in unexplained DD/MR-MCA. We describe a female patient and her maternal aunt, both showing an undiagnosed MCA/MR syndrome, associated with the same subtelomeric rearrangement. Subtelomeric analysis, performed respectively at age 29/12 and 28 years, showed, in

both, distal monosomy 10p and distal trisomy 12p as follows: 46,XX,ish der(10)t(10;12)(p15;p13.2)mat(496A11+,306F7-,137E24+). Parental subtelomeric analysis revealed the proband's mother and the maternal grandmother to have a cryptic balanced 10:12 telomere translocation. Both subjects had IUGR, DD/MR, no speech, hypotonia, lax ligaments. Proband (3 years) showed sparse, curly hair; frontal bossing; sparse eyebrows; blepharophimosis; bilateral ptosis/epicanthus; broad, depressed nasal bridge; low-set, overfolded ears; micrognathia; sacral/coccigeal dimples; puffy hands/feet; long hallux; height at 10°, weight 3°, OFC 2°-50°. Aunt (28 years) showed bilateral epicanthus; beaked nose; short philtrum; thin upper lip; hypoplastic left helix; micrognathia; high palate; crowded teeth; proximally implanted thumbs; concave nails; long hallux; toes/metatarsus valgism bilaterally; puffy feet; sacral dimple; height/weight <3°, OFC <2°. Both show overlapping features with Ohdo syndrome. The phenotypic differences among them appear to be age-related. Such subtelomeric rearrangement has never been reported. 10p- has been described isolated (never as subtelomeric) or as part of different rearrangements. Subtelomeric 12p+ has been reported twice, isolated or as part of a different rearrangement. Our patients show features of partial 10p- rather than partial 12p+. Our observation raises questions about the etiology of Ohdo syndrome.

P0128. Molecular analysis of Stickler syndrome with great interfamilial heterogeneity.

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Stickler syndrome is an inherited connective tissue disorder with ocular, facial and skeletal abnormalities. We report a case of a 4-year-old male. He presented with high myopia, which was first noticed at the age of 12 months, a mild sensorineural hearing loss, midface hypoplasia and arthralgias. He had mild developmental delay. The family history revealed that: 1) the patient's mother had unilateral retinal detachment due to high myopia, 2) The other two patient's siblings had high myopia, 3) The mother's brother also had high myopia, arthritis and slight mental retardation 4) The patient's grandmother and great grandmother (from the mother's side) also suffered from the same high myopia. The patient's x-rays showed mild platyspondyly. The slit lamp biomicroscopy revealed primary membranous vitreous anomaly. The karyotype was normal (46, XY). All the clinical and laboratory tests showed that our patient had Stickler syndrome. To confirm the diagnosis we proceeded to DNA molecular analysis by dHPLC (WAVE). A heterozygous C > T missense mutation c.1486C>T was identified in exon 24 of the COL2A1 gene in all affected members of the family (p.Arg365Cys). Conclusions: Our study showed a high penetrance but great variability in the severity of the symptoms of the affected members of the family. The prompt detection of the mutation at an early age is important for the continuous follow up in order to prevent any of the severe complications that may appear at a later age. It is also necessary for the correct genetic counseling of the family.

P0129. Cytogenetic and chromosome Y microdeletion analysis of infertile men from North-Adriatic region of Croatia

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Microdeletions of the long arm of the chromosome Y and major chromosomal aberrations are common cause of male infertility. One hundred and twenty four infertile and forty control fertile men from North-Adriatic part of Croatia were screened according to the Laboratory guidelines for molecular diagnosis of Y chromosome microdeletions. Microdeletions were determined in two patients in the nonobstructive azoospermia group (27 patients) making the frequency of 7.4%. In the other investigated groups of patients: severe oligoasthenozoospermia (19), oligoasthenozoospermia (44) and normoasthenozoospermia (34) or in the control group, no microdeletions of the long arm of chromosome Y were found. Microdeletion found in one patient was spanning AZFb and AZFc regions, while in the other one was restricted to AZFc region. Cytogenetic analysis was made in 85 patients included in the microdeletion

testing. The overall frequency of chromosomal aberrations was 4.7%. Reciprocal translocation t(12,22) was found in normoasthenozoospermic man (1.2%), and Klinefelter syndrome was determined in three patients (3.5%) with azoospermia. The frequency of Y chromosome microdeletions is within values reported by other authors.

P0130. The influence of three Endothelin-1 (ET-1) polymorphisms on the progression of IGA nephropathy (IGAN)

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Background: The clinical course of chronic renal diseases and their progression to end stage renal failure (ESRF) is highly variable. Different candidate gene polymorphisms, affecting mainly the onset/development of arterial hypertension, have been advocated as possible modulators of the progression. Endothelin-1 (ET-1) has been suggested to be a major disease promoting factor in renal disease. We investigated a possible association of three single-nucleotide polymorphisms of ET-1 K198N, T-1370G and 3A/4A with the progression of IGAN towards end stage renal disease, as well as the clinical and histological manifestations of IGAN.

Methods: We examined a group of 173 patients (pts.) with histologically proven IGAN (99 pts. with normal renal function, 74 pts. with ESRF), as a control group we used 200 genetically unrelated healthy subjects. DNA samples from collected blood were genotyped for three single-nucleotide polymorphisms of ET-1 K198N, T-1370G and 3A/4A by means of polymerase chain reaction (PCR) with defined primers, electrophoresis on 2 % agarose gel and UV light visualisation. We compared the frequencies of different genotypes between the IGAN groups with normal renal function and ESRF.

Results: The ET-1 genotype distribution showed no differences among the groups of IGAN with normal renal function (1.K198N-64,6%KK,31,3%KN,4,0%NN;2.TT-69,1%TT,27,8%TG,3,1%GG;3.3A/4A-45,6%3A/3A,45,6%3A/4A,8,9%4A/4A), IGAN with ESRF (1.K198N-64,9%KK,33,8%KN,1,4%NN;2.TT-76,1%TT,22,5%TG,1,4%GG,3.3A/4A-51,6%3A/3A,35,5%3A/4A,12,9%4A/4A) and control group (1.K198N-62,5%KK,34,5%KN,3%NN,2.TT-76%TT,22,5%TG,1,5%GG,3.3A/4A-51,5%3A/3A,45%3A/4A,3,5%4A/4A).

The distribution of ET-1 genotypes did not differ among IGAN with normal renal function, IGAN with ESRF and control group.

Conclusion: We excluded the effect of K198N, T-1370G and 3A/4A polymorphisms of ET-1 gene on the progression of IGAN to ESRF. Supported by the grant project NK 7733-3, VZ MŠMT 00216 208 06

P0131. SIX3 and ZIC2 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 31 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 7 pathogenic mutations (23%), 5 out of 28 sporadic cases (18%) and 2 out of 3 familial cases (67%). Three mutations were detected in the SIX3 gene and four mutations in the ZIC2 gene. A ZIC2 mutation in a sporadic case appeared not to be a de novo mutation, but was also found in a healthy mother with no clinical features of the HPE spectrum. This was also seen in a carrier of the mutation in a familial case. In this study the genetic heterogeneity of the disease and the extremely variable phenotypes in HPE families have been confirmed.

P0132. The molecular-genetic study of Marfan syndrome in Russia.

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Marfan syndrome (MFS) is an inherited autosomal dominant disorder of connective tissue. Abnormalities appear mainly in skeletal, ocular and cardiovascular systems. The prevalence is approximately 10:100 000 and about 30 % of them are sporadic cases. The prevalence of MFS in Republic of Bashkortostan is 2,5: 100 000. We suppose that such low ratio is probably concerned with insufficient genetic counseling in remote areas: 83% of registered patients live in large cities. We also suppose that the prevalence of MFS depends on ecological background. Because of known associations of MFS with mutations in the FBN-1 gene we have carried out mutations screening of this gene by SSCP method with further direct sequencing. We analyzed 30 exons of FBN-1 gene in 70 patients with MS from different regions of Russia (Republics of Bashkortostan, Kabardino-Balkaria, the North-Osetia-Alania, Sakha-Yakutia). SSCP analysis revealed different abnormal migrating patterns. We identified 2 missense mutations (G1176Y in exon 28 and C2489Y in exon 60) which affect cbEGF-like motifs of fibrillin-1 protein in two patients with classical MFS symptoms. We also revealed 5 mutations which don't lead to amino acid substitutions (IVS15+10delA, 2952C>A in exon 24, 3294C>T in exon 26, IVS61+17InsG, IVS65+13InsG). We identified 4 reported polymorphisms (1875T>C in exon 15, IVS17-46A>G, IVS28+15delTTTA, IVS62+8A>C). Polymorphism revealed in exon 15 (1875T>C) has been found in 24 patients (30,4%).

P0133. Family case-report on the balanced translocation and the monogenic syndrome combination

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A 6-month-old girl was sent to the geneticist's consultation on suspicion of arthrogryposis. During the examination the narrow palpebral fissures, the elbow joint contractures attracted the doctor's attention. The early motor milestones corresponded to the girl's age. The patient's karyotype revealed a balanced translocation: 46,XX,t(1;18)(p10;q10). The family was invited for a thorough examination and the diagnosis specification.

The father's phenotype includes blepharophimosis, ptosis of eyelids, inverted epicanth, arch-looking eyebrows, low-set prominent ears that goes into autosomic dominant syndrome BPES, OMIM:110100, which gene is localized on the 3q23. The intellect has not become worse. Karyotype: 46,XY,t(1;18)(p10;q10).

The elder sibling, a 12-years-old brother, has his father's phenotypic features, such as blepharophimosis, ptosis of eyelids, inverted epicanth, arch-looking eyebrows, low-set prominent ears. The intellect is on the level. Karyotype: 46,XY.

The mother's phenotype has not got any peculiarities. Karyotype: 46,XY.

To sum up, the father exposed the balanced translocation and the monogenic syndrome combination. The son inherited the monogenic syndrome BPES and the daughter -the balanced translocation. At the same time, one can suppose the daughter possesses the incomplete penetration of the BPES. The narrow palpebral fissures and joint contractures are described in the phenotypic features of this syndrome according to the McKusick catalogue.

P0134. Different genetic mechanisms resulting in Prader Willi syndrome - genetic counselling consequences

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Prader Willi syndrome (PWS) is a neurobehavioural disorder arising through a number of genetic mechanisms. All involve loss of paternal gene expression from chromosome 15q11q13. We present two PWS cases with different genetic etiology.

First propositus exhibited severe after birth hypotonia, later on cardiac defect was evaluated. After the first year of age gain of weight and typical appearance of PWS was observed.

Cytogenetic investigation confirmed translocation 46,XX,t(8;15)(q24.1;q21.2). The same translocation was found in the mother. FISH analysis for PWS detection didn't prove deletion of the critical region. Finally uniparental maternal disomy was confirmed.

Second proband exhibits obesity, moderate mental retardation and severe epilepsy. Short after the birth he failed to thrive and was investigated due to hypotonia. The PCR using STR polymorphisms at 15q11q13 showed normal pattern. Finally methylation studies confirmed the diagnosis of PWS, defect of IC centre is suspected.

Although the majority of cases of PWS are sporadic, precise elucidation of the causative genetic mechanism is essential either for the accurate diagnosis as well as for the precise genetic counselling.

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P0135. Two novel TNNI3 mutations in restrictive cardiomyopathy

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Tropomine I (TNNI3) is a sarcomeric protein expressed in the human ventricular myocardium. The protein is essential for the coupling between the myosin heavy chain globular head and actin during contraction of the cardiac fibers. Occasionally mutations in TNNI3 are found in families with hypertrophic cardiomyopathy (HCM). Recently, also some mutations were reported in patients with restrictive cardiomyopathy. Restrictive cardiomyopathy is a rare cardiomyopathic disorder characterized by impaired ventricular filling with reduced volume, ultimately leading to heart failure. Especially in young children the prognosis is poor compared to adults where the clinical course is more variable. In this study we screened two exons of TNNI3, known to contain the majority of previously identified mutations, in 69 HCM families for mutations by DHPCL. In addition, the complete TNNI3 gene was analyzed by direct sequence analysis in four families with idiopathic restrictive cardiomyopathy. No mutations were identified in the HCM families. However, in two of the four unrelated probands with restrictive cardiomyopathy we found three mutations. In proband 1 we found a novel splice site mutation (IVS7+2delT) and in proband 2 we found a known mutation (R145Q) together with a novel mutation (Arg192Cys). In both families the probands were young girls (age 0.5 and 9 years respectively). The disease manifestation is more severe in proband 1, which might be explained by the difference in the underlying genetic defect. These data indicate that TNNI3 should be analyzed completely when restrictive cardiomyopathy is diagnosed especially in young patients.

P0136. Familial stenosis of the pulmonary artery branches with a JAG1 mutation

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We present a case of familial isolated stenosis of the pulmonary artery branches, which was found to represent a mild form of Alagille Syndrome (AGS).

AGS is an autosomal dominant disorder, characterised by intrahepatic cholestasis, heart, ocular, vertebral defects and typical facial features. A mutation in the NOTCH ligand Jagged1 (JAG1) is found in most patients. However, JAG1 mutations have been associated with a wide spectrum of clinical manifestations, from clinically insignificant findings to classical Alagille syndrome, with remarkable intrafamilial variation. The proband, aged 11 months, was diagnosed with mild stenosis of the pulmonary artery branches. His mother had been diagnosed with the same heart defect as a child. No striking dysmorphic features were noted. Karyotype was normal and FISH excluded 22q11 and 10p13 microdeletions.

Although the child had no peculiar facial features, he did have, as his

mother, a prominent forehead and deep-set eyes. Ophthalmologic examination revealed abnormalities of the eye anterior chamber in both. The mother also had minor vertebral findings. Both had normal liver function and abdominal sonography. This, together with reports of *JAG1* mutations in familial isolated congenital heart defects, prompted us to study *JAG1*. Molecular analysis revealed a deletion in exon 9 (1126delG), which is predicted to cause a frameshift leading to a prematurely truncated protein. The mutation was shown to be of maternal origin.

This family represents a case of mild AGS, illustrating the extreme variability of this syndrome, and underlining the importance of facial features in the evaluation of (familial) congenital heart disease.

P0137. Clinical and cytogenetic findings of 38 females with Turner syndrome in Estonia

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Background. Turner syndrome (TS) is a significant cause of growth retardation and/or ovarian failure in females. Patients do not initiate puberty or uterine development. Early cytogenetic diagnosis may improve their quality of life and enables timely hormone treatment.

Objective was a survey of Estonian patients with TS.

Methods. We analysed clinical and cytogenetic data of all females with TS studied by geneticists at Tallinn Children's Hospital during 9 years (1996-2004). In some cases, diagnostic work-ups included FISH and/or chromosomal studies of skin fibroblasts.

Results. We identified 38 females with TS representing three age groups, (Group-1) 16 girls under 12 years, (Group-2) 11 subjects diagnosed from 12 to 17.9 years, and (Group-3) 11 adults diagnosed between 18 and 36 years. Group-1, 75% showed overt TS, 62% short stature under -2SD, 31% discrete anomalies, and 6% delayed puberty. Karyotypes included 45,X (n=14) and mosaics (n=2). Group-2, short stature was present in 81%, overt TS in 36%, and delayed puberty in 36%. Karyotypes included 45,X (n=7), mosaics (n=3), and complex complements (n=1). Group-3, 100% presented amenorrhea, 45% short stature, 36% overt TS, and 9% discrete anomalies. Five patients had karyotypes of 45,X and six showed mosaicism or complex karyotypes. The complex karyotypes will be presented in detail.

Conclusion. TS was suspected based on growth retardation and/or the phenotype in most cases. In 1/3 of the cases, a delayed diagnosis was made based on absent or irregular menstruation.

P0138. Noonan syndrome - clinical evaluation and molecular analysis in 11 portuguese cases

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Noonan syndrome (NS, MIM#163950) is characterized by typical facial features, short stature and congenital heart defect. Its prevalence is estimated 1/1000-2500 new-borns. Familial cases are generally autosomal dominant but neomutations are common. In 2001, the gene PTPN11 encoding for the non-receptor protein tyrosine phosphatase SHP-2, was identified as being responsible for about 30-60% of the cases with SN.

In order to explore the genotype-phenotype correlation, direct sequencing of the exons 3 and 8 of the PTPN11 gene as well as a careful clinical, psychological and laboratorial evaluation was carried out in the Hospital Pediátrico de Coimbra in 11 patients with NS (ten index cases and one with NS/neurofibromatosis type 1 - NF1). Five additional patients with possible NS were also surveyed (two with NS/NF1).

Mutations were found in 2 index cases. In the only familial case evaluated (father and daughter), it was identified a mutation in the exon 8 hotspot N308D (922 A>G). The second case presented a de novo mutation in the exon 3 (D61G; 182 A>G), previously described. We confirmed the lower prevalence of cardiomyopathy in patients with PTPN11 mutations and its absence in the SN/NF1 cases. Both index cases presented atrioventricular canal and chylotorax antecedents, not observed in other patients. The cohort studied had a high prevalence of mental retardation (8/9) as well as other features, likely to result from the referral to genetic units of only the most severe cases.

P0139. Cytogenetic, moleculargenetic findings and syndromes in amenorrhoea etiology

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Primary amenorrhoea (PA) and amenorrhea caused by premature ovarian failure (POF) are heterogeneous groups of menstrual disorders where a genetic basis are suggested.

Purpose. To determine the role of genetic factors in patients with menstrual disturbances.

Material and methods. Clinical and cytogenetical data of 56 PA and 22 POF patients were studied in Genetic Service of Outpatients Children Clinic in the past 10 years (1995-2004). Clinical data and standard karyotypes using G banding were available in all cases, in some cases chromosomal study from skin fibroblasts was performed for final diagnosis. In POF patients premutations in FMR1 gene were detected.

Results. Normal female karyotype occurred in 71% (40/56) patients with PA. The variable forms of Turner syndrome [45,X; 46,XX/45X; 46,Xi(Xq)] were diagnosed in 13 cases. Two patients represents 46,XY gonadal dysgenesis. In 5 female Mayer-Rokitansky-Kuster anomaly and in one MURCS association were diagnosed. Among POF patients normal female karyotype was detected in 68% (15/22) females. Turner syndrome (45,X; 46,XX/45,X) was estimated in two patients. X;autosome translocation 1/22, dicentric X-chromosome 1/22 and Poland syndrome 1/22 were possible causes of secondary amenorrhoea. Two POF patients showed premutation in FMR1 gene.

Conclusion. Our data confirmed the heterogeneous etiology of amenorrhoea. The chromosomal abnormality value is quite high in both groups of amenorrhoeic patients.

P0140. An unknown case report

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An unrelated couple were referred to Medical - Genetic center of Isfahan welfare organization due to their son's problems. The man was 59 years old, and an uneducated worker. The woman was 45 years old an uneducated housewife.

This son is fifth child of his parents. He was delivered normally by N.V.D and was good until 11 month's old. After that time some parts of his body grew more than other parts and became hypertrophic.

According to evaluation from 11 month's old until several years the diagnosis was made as local hypertrophy + Neurofibromatosis. But now which he is 19 years old he hasn't any signs and symptoms of Neurofibromatosis or willm's tumor. He has asymmetric face, abnormal curve in cervical and dorsal spine, high patchy hypertrophy in many parts of his body including some fingers, toes, face, and Because of unusual growing of his right knee, he was operated and his knee was fixed .The report of pathology was proliferation on hyaline cartilage which has resulted in hypertrophy of joint cartilage and possibly of meniscus. There is no evidence of malignant change. Sonography of kidneys and Adrenals are normal. The reports of his radiographies are: sever cervical and dorsolumbar scoliosis with congenital vertebral fusion in cervical spine + L 3/4 spondylolisthesis. RT knee ankylosing + Atrophy + osteopenia, RT femoral neck widening, T9 wedge vertebra. His intelligent is good. He finished his high-school when he was 18 years old.

So now what is your diagnosis?

P0141. Phenotypic differences in Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder affecting several systems caused by a heterozygous deletion in the chromosomal region 7q11.23. A common interval that includes up to 17 genes reported so far is deleted in the great majority of patients. Specific contribution most of deleted genes to the WBS phenotype

remains unknown. WBS is transmitted in an autosomal dominant manner. Most of the cases arises *de novo*, but occasionally parent-to-child transmission is observed. Over 99% of individuals with the clinical diagnosis of WBS have this contiguous gene deletion, which can be detected using fluorescent *in situ* hybridization (FISH). The WBS phenotype is variable, and no single clinical feature is required to establish the diagnosis.

We investigated 13 patients with a clinical diagnosis of WBS or WBS-like features, aged 4 months - 24 years (average 5.5 years). Deletion at 7q11 was found in all patients.

All (100%) patients showed the typical dysmorphic signs, in most cases (11 of 13 = 77%) had some degree of mental retardation (usually mild mental retardation). 11(77%) patients had cardiovascular malformation, 7(56%) had a specific heart anomaly (supravalvular aortic stenosis), 2(15%) none. Growth deficiency and mild microcephaly was observed at 9(69%) patients. Hypercalcemia was observed at 4 (31%) patients.

Our preliminary results are in accordance with the published data. To determine the extend of deletion molecular-genetics analysis using MLPA is in our Institute in progress. This analysis will enable us to evaluate precise phenotype-genotype correlation.

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P0142. Clinical and MRI features of patients with Shah-Waardenburg syndrome associated with SOX10 mutations: a case report and review of the literature

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Shah-Waardenburg syndrome is a rare congenital disorder with variable clinical expression, characterised by aganglionosis of the rectosigmoid (Hirschsprung disease), and abnormal melanocyte migration, resulting in pigmentary abnormalities and sensorineural deafness (Waardenburg syndrome). Mutations in the EDN, EDNRB and SOX10 genes can be found in patients with this syndrome. SOX10 mutations are specifically associated with a more severe phenotype called PCWH: peripheral demyelinating neuropathy, central dysmyelinating leukodystrophy, Waardenburg syndrome, and Hirschsprung disease.

Neuronal expression of SOX10 occurs in neural crest cells during early embryonic development and in glial cells of the peripheral and central nervous systems during late embryonic development and in adults. The natural history of patients with SOX10 related Shah Waardenburg syndrome or PCWH is still unknown. We present a 4-year-old girl with a *de novo* nonsense mutation (S384X) in SOX10. Main clinical features were mental retardation, peripheral neuropathy, deafness, Hirschsprung disease, distal arthrogryposis, white hairlock, and growth retardation. She presented with hypotonia, developmental delay, reduced peripheral nerve conduction velocities, and radiologically assessed central hypomyelination. Subsequently formation of abnormal myelin within the central and peripheral nervous system was functionally and radiologically assessed.

Children presenting with features of Waardenburg syndrome and neurological dysfunction should be tested for mutations in the SOX10 gene to enable diagnosis and counselling.

P0143. A three generation family with dominantly inherited coloboma associated with a nonsense PAX6 mutation

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The PAX6 gene, a member of the paired box family, encodes a transcription factor. PAX6 is involved in eye morphogenesis and is expressed in the developing central nervous system and numerous ocular tissues during development. Autosomal dominant aniridia was the first condition to be associated with PAX6 mutations. Since then PAX6 mutations have been detected in various ocular anomalies

including Peters' anomaly, corneal dystrophy, congenital cataracts and dominantly inherited nystagmus, foveal hypoplasia, and a variety of optic nerve malformations. There is no clear cut phenotype-genotype correlation in ocular defects related to PAX6 mutations nevertheless nonsense mutations are predominantly associated with classical aniridia.

Herein we report a three generation family with dominantly inherited coloboma showing a nonsense PAX6 gene mutation, R203X. Within the family the phenotypic spectrum of the typical coloboma and ocular malformation is wide, illustrating an intrafamilial variability of expression.

Previously the 15 recorded cases of this nonsense mutation have been associated with the aniridia phenotype. Furthermore the typical coloboma phenotype caused by PAX6 gene mutations has only been reported three times, and in each case never showing a nonsense mutation.

P0144. Prenatally Detected Extra Structurally Abnormal Chromosome: a Dilemma for Genetic Counseling

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We describe a series of 3 cases with prenatally detected Extra Structurally Abnormal Chromosome (ESAC). All were of spontaneous conceptions. The first was singleton, the second and third were ones of twins, bichorionic diamniotic and monozygotic respectively. In two cases amniocentesis was performed due to advanced maternal age and the ESAC was a co-incident finding and in the third, amniocentesis was performed due to increased Nuchal Translucency in one twin. Utilizing Fluorescent *In Situ* Hybridization (FISH), ESACs were shown to be bisatellited and were derived from chromosome 15 in cases 1 and 2, inv dup (15), and monosatellited that derived from chromosomes 14/22 in case 3. The ESAC was maternally transmitted in case 2 but was *de novo* in cases 1 and 3. No mosaicism was detected in the first two cases but a level of 80% was demonstrated in the third. FISH to the Prader Willi region in the 15-ESACs and Cat Eye Syndrome region in the 14/22-ESAC showed no signal. In all cases uniparental disomy was excluded. Following an extensive genetic counseling, pregnancy 1 was terminated while the other two pregnancies were continued. The decision making process particularly of monozygotic twins with hetero karyotypes is presented.

P0145. Two novel families with Borjeson syndrome ascribed to a recurrent PHF6 gene mutation

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A diagnosis of Borjeson syndrome was made in two families. The affected persons were 2 brothers in family 1, and 3 males (a young boy and his 2 uncles) and a woman (half sister of the 2 uncles) in family 2.

In both families, diagnosis was made on clinical grounds for the 3 young boys. They had a similar presentation with intra uterine growth retardation and neonatal hypotonia. Developmental retardation became rapidly obvious, later with hyperactivity. They had feeding problems with failure to thrive. Nevertheless, after the age of 3 they developed obesity and hyperphagia. Facial gestalt suggested the diagnosis of Borjeson syndrome : we considered that the shape of their mouth and their ears is particularly noteworthy.

3 of the 4 conducting women were totally asymptomatic. X inactivation was 100% skewed in these 4 conducting women.

Other persons with mental retardation had been further identified in these 2 families and will be studied.

The coding exons of the PHF6 gene were screened by DHPLC and the sequence of exon 10 revealed a nucleotide substitution conducting to the R342X nonsense mutation. This mutation has previously been described in 3 families, including the original family described by Borjeson.

We therefore confirm the recurrent nature of the R342X mutation of the PHF6 gene in our 2 independent families.

Our observation further emphasize the possible expression in woman in spite of a totally skewed X inactivation.

P0146. Three novel *CDKL5* mutations in patients with a severe early onset seizure disorder and phenotypic overlap with Rett Syndrome.

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Objective

The *CDKL5* gene has been implicated in infantile spasms in two females with X;autosome translocations. Recently, we described further mutations in two families with a Rett Syndrome (RTT)-like phenotype. We aimed to determine the frequency of *CDKL5* mutations in different subtypes of RTT.

Methods

The coding region of *CDKL5* was screened by DHPLC and/or direct sequencing in 94 patients initially referred for *MECP2* analysis :16 males and 78 females, of whom 38 fulfilled the criteria for RTT and 33 had seizures in the first year of life. All were negative for *MECP2* mutations both by sequencing and quantitative analysis of exons 1, 2, 3 or 4.

Results

We identified 3 novel, pathogenic mutations in *CDKL5*. Two of these were splice site mutations, confirmed by further RNA studies. One was a de novo missense change in a conserved region of *CDKL5*. Two patients presented with an epileptic encephalopathy and one with RTT. All had seizures in the first 3 months of life. The phenotype will be discussed in the context of the other reported cases, and tentative genotype-phenotype correlations made.

Conclusions

This study reinforces the observation that the *CDKL5* phenotype overlaps with RTT. *CDKL5* mutations are relatively common in patients whose seizures begin before the age of 6 months (3/17 in this study). *CDKL5* analysis is indicated in patients with features of RTT and a severe seizure disorder commencing in the first 3 months of life or infantile spasms. In addition it may be of value in undiagnosed epileptic encephalopathy.

P0147. A new autosomal recessive form of congenital extraocular muscular fibrosis with associated malformations and exclusion analysis of ARIX gene

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Congenital extraocular muscle fibrosis (CFEOM, MIM 135700) is an inherited strabismus characterized by restrictive ophthalmoplegia. To date, a total of three loci on chromosomes 12q12 (FEOM1), 11q13 (FEOM2) and 16q24 (FEOM3) were reported. Mutations in KIF21A (12q12) and ARIX (11q13) genes are responsible for both autosomal dominant (AD) and recessive (AR) forms respectively. No gene has yet been identified for 16q linked families demonstrating AD inheritance with reduced penetrance and variable expressivity. Here, we report a nuclear pedigree with two affected and a normal sib born in a consanguineous parents. One affected member had restrictive exotropia with the globes frozen in abduction, and a normal levator function. The other subject demonstrated unilateral ptosis with contralateral loss of ocular movements. Both patients had a short stature, unusual facies, contractures of extremities, camptodactyly, pes cavus, patellar hypoplasia, and scoliosis. Electroneuromyography (ENMG) revealed normal neural motor and sensorial conduction velocities. This family tested for a linkage to AR-FEOM2 locus. The order of the markers with respect to ARIX gene are: D11S2006-(8.9Mb)-D11S4113-(1.66Mb)-D11S4139-(1.45Mb)-ARIX-(1.72Mb)-D11S4207/D11S2371-(6.29Mb)-D11S2002. Entire region was excluded by lack of homozygosity and no significant linkage. Systemic malformations such as craniofacial

dysmorphism, dental anomalies, scoliosis and flexion contractures were previously described in few sporadic cases and no hereditary component has yet been reported. This family presenting AR-CFEOM associated with other system malformations and lack of linkage to ARIX region represents a new form of CFEOM phenotype (CFEOM4)

P0148. Raine syndrome : report of a new case

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Raine syndrome is a rare lethal osteosclerotic bone dysplasia, first described in 1989. It associates generalized osteosclerosis and craniofacial dysplasia.

We report a new case in an infant girl born to consanguineous Moroccan parents.

Multiple fetal anomalies were detected on ultrasonography during pregnancy :

microcephaly with craniostenosis and dysmorphic features including midface hypoplasia, exophthalmos, hypoplastic nose, low-set ears. Intracranial calcifications and hydranmios were also present.

Fetal karyotype on amniotic fluid cultures was normal (46,XX).

At birth, antenatal findings were confirmed and additional features included cleft palate, hypertrophic gums, hirsutism and generalized osteosclerosis. The baby died shortly after birth of respiratory distress due to lung hypoplasia.

Diagnosis of Raine syndrome is based on two major features : typical craniofacial dysplasia and generalized osteosclerosis. Prenatal diagnosis has not been reported for a first case but would be possible when confronted with the association of intracranial calcifications and characteristic craniofacial features. To date only 13 cases have been reported in the literature. Autosomal recessive inheritance has been suggested, but the underlying metabolic or molecular defects are not yet known.

P0149. Wilkie Oculo-Facio-Cardio-Dental (OFCD) syndrome

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Wilkie Oculo-Facio-Cardio-Dental (OFCD) syndrome is a rare MCA syndrome with congenital cataract, microphthalmia, secondary glaucoma, ptosis, characteristic face, atrial/ventricular septal defect, cleft palate, dental and digital abnormalities. The facial dysmorphia consists in a long and narrow face, broad nasal tip, which appears bifid and long philtrum. The dental abnormalities include hypo/oligodontia, radiculomegaly, delayed tooth eruption and dental fusion. OFCD is an X-linked dominant condition with presumed male lethality and probable skewed X inactivation in female carriers. It results from specific mutations in the *BCOR* gene, located on Xp11.4, which is a key transcriptional regulator during early embryogenesis, particularly in the eye, skeleton and central nervous system. Mutations in *BCOR* are also found in MAA2 (microphthalmia with associated anomalies type 2), one form of Lenz microphthalmia.

We report the case of a 14 year old girl, who presented with bilateral congenital cataract, secondary glaucoma of the right eye, oligodontia, dental fusion and mild developmental delay. She had a long face, characteristic nose shape, asymmetric eye globes with right microphthalmia, a nasal speech. Her dental examination showed fused medial and lateral upper incisors, large interdental space, and agenesis of the medial lower incisors. The diagnosis of OFCD syndrome has been proposed, despite the absence of heart defect and mild developmental delay. The molecular analysis of *BCOR* is still underway at time of submission.

P0150. A boy with facial dysmorphism, macrocephaly, and Dandy-Walker malformation due to familial unbalanced traslocation der(18)t(11;18)(q23.3;p11.21)mat

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The proband is a 14-years old boy born after the fifth normal pregnancy and delivery, born at term, in a family of young and unrelated Caucasian parents. Birth weight and length were 3300g and 52cm, respectively. He was referred to the clinic at 1 year and 7 months of age because of facial dysmorphism, cleft soft palate and developmental delay. He presented with macro-dolichocephaly, occipital encephalocele, dysmorphic facies, high prominent forehead, hypertelorism, ptosis, protruding eyes, convergent strabismus, prominent philtrum, down-turned mouth corners, high palate, dysplastic ears, generalised hypotonia, hypoplastic genitalia with bilateral cryptorchidism, club feet and mental retardation. CT scan revealed a Dandy-Walker malformation. During his early childhood the diagnosis of Walker-Warburg syndrome was considered even the absence of specific ocular abnormalities.

Clinical evaluation at 14 years of age revealed a distinct phenotype consisting of macrocephaly, peculiar face, prominent dysplastic ears, triangular mouth, generalized hypotonia, hypoplastic genitalia and cryptorchidism. Family history was positive for recurrent spontaneous miscarriages and perinatal deaths with multiple congenital anomalies. G-banded high-resolution re-examination of the karyotype revealed an unbalanced maternal translocation between chromosomes 11 and 18 resulting in partial trisomy 11q23.3→qter and partial monosomy 18pter→p11.21. Additional molecular cytogenetic analysis was performed to unravel the phenotype/genotype correlation in this unique patient.

P0151. Genotype-phenotype relationships in Noonan syndrome

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Noonan Syndrome (NS) is an autosomal dominant disorder, characterized by short stature, typical face and congenital heart defects. In approximately 50% of cases the condition is caused by missense mutations in the *PTPN11* gene on chromosome 12, resulting in a gain of function of the protein SHP-2.

In this study *PTPN11* mutation analysis was performed in 170 unrelated NS patients. In 76 (45%) of them a mutation was identified. In total 24 different heterozygous missense mutations were detected; 48.7% were located in the N-SH2 domain, 48.7% in the PTP domain, and 2.6% within the C-SH2 domain. The 922A>G change was most often detected (21%). The benefit of the NS scoring system developed by van der Burgt et al. [1994] is shown: among physicians who based their diagnosis on this NS scoring system the percentage mutation positive subjects was 54%, whereas this percentage was only 39% among the others. Of the 56 well documented mutation-positive NS patients, 38 patients (68%) had a valvular pulmonary stenosis, 4 patients (7%) had hypertrophic obstructive cardiomyopathy, and 14 patients (25%) had no heart defect. Two patients with classical NS and some uncommon manifestations had a mutation in the C-SH2 domain. A trend was observed in patients carrying the 922A→G change (Asn308Asp) receiving normal education.

In one patient with NS and mild juvenile myelomonocytic leukemia (JMML) the mutation 218C→T (Thr73Ile) was found. This confirms previous findings indicating that individuals with NS with specific mutations in *PTPN11* are at risk of developing JMML.

P0152. Trisomy 21 presenting as fetal heart rhythm anomalies

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Objective: To describe the clinical case of a neonate with Trisomy 21 associated with heart rhythm anomalies. **Case Presentation:** A male newborn was delivered at 36 weeks gestational age by emergency cesarean section for fetal bradycardia. The clinical examination of the newborn noticed a Down phenotype and bradycardia (40/minute). The electrocardiogram showed an aspect of total atrioventricular block

with idioventricular rhythm. The heart ultrasound showed an Atrial Septal Defect of type ostium secundum, together with atrio-ventricular asynchrony. There were not associated other organ malformations. The block was not responsive to medical therapy and the patient died in the third day of life due to heart failure. **Conclusion:** This is an unusual form of presentation of a Trisomy 21 with a bad prognosis. This etiology should be taken into account in the differential diagnosis of the fetal bradycardia.

P0153. The 22q13.3 microdeletion syndrome: description of a case with a particular phenotype

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The first case of 22q13 deletion syndrome was identified by the results of cytogenetic analysis in a family with pericentric inversion of chromosome 22. Approximately 70 patients with this syndrome have been reported and the clinical phenotype is now better delineated. We describe the case of a child, 8 years old, second daughter of non consanguineous parents, born at term from caesarean section performed in reason of macrosomic foetus. At birth a wide cheilognathopatatoschisis was diagnosed. After birth the newborn suffered from anoxia and hypoglycaemia. The proband was able to walk autonomously at two years of life. At the moment she pronounces only senseless sounds. In infancy several feverish seizures happened and antiepileptic therapy (valproic acid) was started. Clinical examination showed "Kabuki-like" phenotype with long palpebral fissures, sparse lateral eyebrows, convergent strabismus, hypertelorism, large ears with "cup-like" aspect, thick lips, occipital exostosis, long fingers and toes, bilateral syndactyly between II-III toes, overgrowth, diffuse hypotonia and severe mental retardation. The proband previously performed: standard karyotype (46 XX), diurnal and nocturnal video-EEG polygraphic registration (absence of sleep physiological pattern); cerebral MRI showed hypoplasia of corpus callosum and dysmorphic aspect of cerebellar vermis, amigdalas and IV ventriculus.

A cryptic 22q13.3 deletion was detected by FISH analysis, using the VCFS/DGS FISH Kit (Oncor USA).

Conclusion: we are now planning a deletion study at molecular level, to determine the size of the 22q13 deletion, in our patient, using minisatellite, microsatellite and FISH analysis, and to get phenotype/genotype correlation informations, comparing with the literature data.

P0154. Singleton-Merten Syndrome: Evidence of autosomal dominant inheritance in the first European family

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In 1973, Singleton and Merten described two unrelated females with dental dysplasia, aortic valve calcification, glaucoma and widened medullary cavities of the phalanges. Up to now, only 2 consecutive case reports have been published, including one Canadian family with multiple affected family members of both sexes.

A 28 year old German woman and her 23 year old brother presented with juvenile glaucoma, calcifying aortic valve stenosis, dental dysplasia with poor root development, acro-osteolysis of the hands and feet, widened medullary cavities of the metacarpals and phalanges and focally decreased bone density. In the female, the aortic and mitral valve had to be replaced because of extensive calcifications at the age of 14 and 28 years, respectively. Both siblings had no pathogenic mutation in *ENPP1*, a gene recently linked to generalized infantile arterial calcification. Serum levels of receptor activator of NF- κ B and osteoprotegerin (which, when deleted in mice, are associated with osteoporosis and vascular calcification) were normal. In the male proband, *FBN1* sequencing analysis did not reveal a pathogenic

mutation. Family history revealed vertical transmission of the phenotype from the father, who lost his secondary dentition before the age of 18 and developed intracardiac calcifications at the age of 39 years. This study presents the first European family with Singleton-Merten Syndrome and confirms autosomal dominant inheritance with variable expression. The finding of focal osteoporosis, dental dysplasia and cardiovascular calcifications could point to an inborn defect of an extracellular matrix protein regulating bone resorption and soft tissue calcification.

P0155. Coffin-Siris syndrome - a case with macrocephaly and anus displacement

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Aim: to present a new case of Coffin-Siris syndrome, a rare genetic disorder, characterized by the typical facial phenotype with absent/hypoplastic nail and terminal phalanx of the fifth finger. The mode of inheritance is thought to be autosomal recessive, although majority of affected individuals are female (85%).

Case report: a female infant, the first child of healthy parents, born after vaginal delivery. At birth body weight, length and head circumference was normal but lumbosacral hypertrichosis, umbilical hernia and anus displacement were noted. Her psychomotor development was delayed and accompanied by hypotonia, feeding difficulties and recurrent upper respiratory tract infections. Physical examination at the age of 4 showed: macrocephaly with sparse scalp hair, flat nasal bridge with wide nasal tip, upturned nostrils and wide mouth. Moreover hypoplastic fifth digit nails and toenails were noted. She had expressive language delayed and was very irritable. X-ray of the hands shows absence of terminal phalanx of the fifth fingers. No other abnormalities on MRI of the brain and internal organs were noted. Metabolic testing for mucopolysaccharides excretion was within normal range.

Discussion: The clinical features show typical stigmata of Coffin-Siris syndrome with: "coarse face", absence of the distal phalanx of the fifth finger and developmental delay. The presence of macrocephaly and anteriorly placed anus has not been described previously. Thus, this new clinical features further expanded the symptomatology of Coffin-Siris syndrome.

P0156. Prader-Willi syndrome - 5 years diagnostic experiences in prepubertal children in Slovakia.

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Prader-Willi syndrome (PWS) is one of the most common recognized genetic forms of obesity due to loss of paternal genes located in the 15q11-13 region which spans a large cluster of imprinted genes. The disorders are caused by four genetic defects (microdeletion, single gene mutation, uniparental disomy, imprinting defects) and for their detection different laboratory methods are required. In this study we summarise clinical data on 13 Slovak PWS patients which were laboratory confirmed in prepubertal age during the last 5 years. This group includes 10 boys and 3 girls with a mean age of 6.5 years. New PWS cases have been recently diagnosed in the first year of life, including one newborn. The PWS score in our patients ranged from 5 to 10 points. Except for 3 children, microdeletion was detected by use of FISH methods. DNA analysis with a specific methylation probe confirmed presence of only the maternal methylation pattern in 2 cases and uniparental maternal disomy was observed in one case. Management of PWS patients including growth hormone substitution may have a positive effect on the health, functional abilities and longevity of affected individuals (Einholzer et al., 1998; Cassidy, 2001). This depends on early diagnosis of PWS confirmed by laboratory tests. Our results during the last five years reflect good coordination of continuing genetic education and close cooperation with endocrinologists and other medical specialists.

P0157. Fragile X FMR1 triplet repeat detection by PCR

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Fragile X syndrome is the most common cause of inherited mental retardation. The causative mutation in 99% of cases is full expansion of the CGG repeat in the 5' untranslated region of *FMR1* (fragile X mental retardation), which is located on the X chromosome. Full mutation is associated with hypermethylation and absence of gene product, FMRP (fragile X mental retardation protein). Genetic testing for fragile X syndrome consists of identifying the CGG repeat length: normal size, 5-44 repeats; intermediate-grey zone, 45-54 repeats; pre-mutation, 55-230 repeats; full mutation, greater than 230 repeats. Carrier females and newborns with larger than normal repeats are difficult to detect with PCR due to disproportionate amplification of repeat sizes. Celera Diagnostics has developed research PCR reagents capable of amplifying more than 680 repeats. An ABI Prism® 3100 Genetic Analyzer research protocol was developed that accurately sizes normal repeats within one repeat and pre-mutation repeats within three repeats. Inclusion of a sex-linked gene that co-amplifies in a multiplex with the triplet repeat allows gender and X chromosome number determination. Homozygote normal females with repeats of identical size can be identified and carrier females with large repeat size differences can be flagged for reflex testing. The AB 3100 research protocol can detect up to 117 repeats while keeping small repeats on scale and maintaining normal allele copy number determination. We have developed an efficient and robust PCR method for rapid and accurate detection of normal and pre-mutation CGG repeats in the *FMR1* gene.

P0158. Molecular and cytogenetic analysis in IVF failure cases

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Chromosomal abnormalities in infertile couples results in spermatogenic arrest, premature ovarian failure, implantation failure and consequently failure of *In Vitro* fertilization (IVF). Thirty eight infertile couples with IVF failure having poor blastocyst development and implantation were analysed cytogenetically and for molecular analysis of AZF loci in the men.

Two females with recurrent IVF failure showed partial deletion of long arm of one of the X chromosomes and the other female had 10% cell line showing deletion of pericentromeric region of long arm of chromosome number 1. The male partners in these cases were cytogenetically normal and had no microdeletion in the AZF loci. Of these couples microdeletion analysis of 30 cytogenetically normal infertile men, only two cases showed deletion; one with AZFc loci (STS deleted sY 254,sY255) and the other case had deletion of AZFb loci (STS deleted sY127,sY134).

The couple where female partner had deletion of long arm of X chromosome(Xq-) resulted in repeated failure of blastocyst development, in 4 IVF cycles. The case with AZFb microdeletion had maturation arrest and case with AZFc deletion had hypospermatogenesis. In these cases sperms could be retrieved from the testis, to be used for IVF or Intracytoplasmic sperm injection. (ICSI). Thus AZF analysis determines the prognosis and management of these cases. 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.

P0159. Prevalence of hereditary pathology in infants with congenital malformations in Moldova

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The present study deals with the data of medical genetic investigation of 2594 infants with congenital malformations (CM) born in the period 1990-2000 ascertained by population-based monitoring CM in newborns in Moldova.

The most frequent were MC multifactorial inheritance - 45.2% cases and included most isolated MC involving the heart - 19.8% cases,

musculoskeletal system - 26.1% cases, central nervous system - 10.9% cases. Autosomal recessive pathology was in 5.1%, included 34 nosological forms with 133 infants. Parents were consanguineous in 11.3%. Autosomal dominant pathology was seen in 6.4%, included 32 nosological forms with 165 patients. 83.6% of cases were sporadic, determined by mutation *de novo*. X-linked diseases included 9 syndromes and constituted 0.5% X-linked recessive pathology and 0.2% X-linked dominant pathology in common structure. Associations were seen in 2.2% and comprised 11 nosological forms (54 infants). Chromosomal abnormalities were found in 11.2%. Major chromosomal abnormalities were Down syndrome - 84.6% cases, Patau syndrome - 0.7%, Edwards syndrome - 0.5%, Turner syndrome - 3.1%, Klinefelter syndrome - 0.3%, structural aberrations - 8.1%, disorders of sexual differentiation and development - 2.7% cases. CM of teratogenic aetiology were established in 5.8%. The most frequent was fetal alcohol syndrome - 39.1%, infection embryofetopathies - 37.8%. The non-classified complex of multiple MC consisted of 23.4% cases.

P0160. Microdeletion 2q31- redefining the correlated clinical phenotype

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Several patients with an interstitial deletion of chromosome 2q have been described till now. The clinical phenotype consists of a variety of digital anomalies of hands and feet, including a wide cleft between the first and second toes, variable toe syndactyly, wide halluces and a spectrum of finger abnormalities, ranging from monodactylous limbs, through brachydactyly type E and D to camptodactyly. In addition there are different internal organ anomalies affecting the CNS, heart and uro-genital system. Hemizygosity for the HOXD13 and EVX2 genes have been proposed to be responsible for the clinical spectrum. Recently, based on the phenotype in patients with overlapping interstitial deletions of the 2q31 region, a new locus responsible for cleft foot-hand syndrome has been mapped proximally to the HOXD cluster between EVX2 and marker D2S294. A 4-year-old boy born to healthy, unrelated parents, presenting with microcephaly, complex CNS anomaly, hypotonia, cleft palate, camptodactyly, 4-5 toe syndactyly, short stature and mental retardation was referred for diagnostic evaluation. Karyotyping revealed an interstitial deletion 2q (23.4-3.1). Combining the deletion data in this patient with the small deletions within HOXD13 in other patients and the literature review, we reinforce the hypothesis that the variable phenotype in patients with an interstitial deletion of chromosome 2q31 is a result of hemizygosity for HOXD gene.

P0161. The case of combination of chromosomal anomaly with oral-facial-digital syndrome, type 1

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We observed a female with combination of chromosomal anomaly (mosaic form of Turner's syndrome) and monogenic syndrome (oral-facial-digital syndrome type 1). Chromosomal anomaly was revealed primary. The cause of cytogenetic study was three miscarriages in her anamnesis. Moreover she had several slightly expressed phenotypic features such as short stature, hypertelorism of eyes, brachydactyly and camptodactyly, polycystic kidneys (without any clinic symptoms). Karyotype was mosaic: 45,X/46,XX; 45,X met in 15% of cells. So phenotype was explained by this chromosomal abnormality. During the next pregnancy the fetus's karyotype was defined and it was normal (46,XX). The patient completed a full-term pregnancy without complication. The newborn girl had multiple congenital abnormalities: midline cleft of upper lip, clefts of maxillary alveolar ridge, multiple hyperplastic oral frenulae, lobulated and bifid tongue, prominent milia on the face, sparse hair, brachydactyly and clinodactyly, partial syndactyly. The child's phenotype absolutely corresponded to oral-facial-digital syndrome, 1 type. The following thorough examination of the mother revealed very small bifid of the tongue and multiple oral frenulae. So the woman demonstrated partial expression of the syndrome and some main features of it were not noticed, especially after revealing of chromosomal anomaly, which seemed to be the only cause of her phenotype. It is unknown if there is connection between

X-linked dominant syndrome, its not severe expression in phenotype and loss of X chromosome in the part of cells. Until this case we have met only one description of combination in female of sex-chromosome anomaly (47,XXX) with oral-facial-digital syndrome.

P0162. A two months old girl with the new autosomal recessive syndrome with Zellweger-like manifestations

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Zellweger syndrome is an autosomal-recessive disorder associated with peroxisomal absence and characteristic phenotypic features: high forehead, broad nasal bridge, epicanthal folds, upslanting palpebral fissures and micrognathia, combined with severe hypotonia, hepatomegaly, renal cysts and developmental delay. This phenotype is characterized with elevation of very long chain fatty acids.

We present a two months old girl with phenotypic features that are typically seen in Zellweger syndrome: high forehead, broad nasal bridge, epicanthal fold, upslanting palpebral fissures, and micrognathia. In addition to the physical anomalies she has also severe psychomotor retardation, hypotonia and multifocal seizures. The hepatic and renal size and structure, very long chain fatty acids and plasma acids level were normal. The child is born from healthy non-consanguineous parents who have an older healthy son.

Ahn and al.(2003)described two sibs born to Ashkenazi Jewish consanguineous parents with similar dysmorphic feature and normal VLCFA. They found respiratory chain abnormalities in muscle biopsy and suggest that this is a new autosomal recessive syndrome that could be due to a nuclear-encoded mitochondrial defect.

P0163. Is there genetic heterogeneity in Classical Hemimelia? An ongoing study in a large UAE national family.

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The Arab population provides unique opportunities to identify and understand the genetics and biology of human traits and their variation. We have come across a very rare form of skeletal dysplasia in a multiple generation UAE national family, which is closely akin to a partially defined genetic disorder known as Tibial Hemimelia (THM). At present, even the gene for the classical form of Tibial Hemimelia (MIM 275220) is not defined. In this UAE national family, an initial analysis of six generations has identified 13 affected persons (both sexes) with a spectrum of skeletal anomalies. Of these, at least 10 are transmitted through a single parent. The ongoing study of some of the members of this family has revealed variable expression, skipped generations, incomplete penetrance, unilateral tibial hemimelia, bilateral tibial hemimelia, foot deformities, post-axial polydactyly (incomplete), syndactyly, oligodactyly and facial dysmorphisms. One of the main skeletal abnormalities is Tibial Hemimelia. We will be analyzing whether this family represents a clinical heterogeneous form of previously defined Tibial Hemimelia or a new entity. The ongoing studies we are pursuing in this family include FISH studies for 8q and 10q regions and a genome wide scanning for the candidate gene using micro satellite markers and SNPs. Identification of the candidate gene in this family will be very helpful for carrier detection, prenatal and postnatal diagnosis.

P0164. The Spanish Overgrowth Syndrome Registry

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The Spanish Overgrowth Syndrome Registry (SOGSR) has been established in 2003 aimed to record patients with Overgrowth Syndromes (OGS). It is located at the Hospital Universitario La Paz, in Madrid, Spain

All patients with OGS are included in a database. Most of these patients have laboratory tests for syndromes and in the majority of them biological samples (DNA; slides, tissues) have been stored. In addition, a clinical picture/s is also requested to document the files. Written permission for both clinical photographs and biological samples are obtained.

The aims of the registry are: -to include all patient with any OGS in a

National database, -to compare frequencies of OGS; -to describe rates of incidence and prevalence; -to monitorise the outcome of associated malformations; -to develop guidelines and algorithms for clinical and cancer follow-up; -to known the frequency and outcome of mental retardation in the OGS; -to collaborate in the clinical, cytogenetic and molecular diagnosis of this syndromes; -to coordinate the molecular tests of each OGS performed in Spain and to create a site and a discussion forum for both physicians and patients or parents.

The addresses are : www.overgrowthsyndrome.org and www.sindromedesobrecrecimiento.org

The Registry is organized in 4 main items. 1- management of the Registry, 2- ILaboratory and the handling of the biological samples, 3- Genetic Counselling and Prenatal Diagnosis, and 4- Projects and Research

By January 2005, 196 patients have been included in the Registry, most of them with Beckwith-Wiedemann, Sotos, Simpson Goalbi Behmel, Macrocephaly -cutis marmorata, Costello syndrome and others.

P0165. MOMO syndrome or new syndrome: description of a case

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Obesity in genetic syndromes is a very common finding, but often it's a part of complex clinical picture and remain undiagnosed.

We describe the case of a child, second daughter of non consanguineous parents, with severe obesity, diffuse hypotonia and serious psychomotor retardation. She began to sit alone only after 12 months and at 5 years she spoke only few words. Since the second year of life an increasing appetite and consequent weight gain were observed. She suffers of recurrent asthmatic bronchitis. At age 7 years she was admitted to our observation. The girl has coarse round face, hypertelorism, bilateral epicanthal folds, large open mouth, lowset ears, short neck, adipomastia, tapering fingers, genua valga and bowing tibiae, striae distensae at the roots of lower limbs. Anthropometric data: height 122cm (+0.58 SDS); family target height 162,5cm (+0.05 SDS). OFC 54 cm (+2.1 SDS). Weight 69.6 kg (>> 99° centile). BMI 46,7 (+24.1 SDS); waist circumference 94 cm; WHR 0.81; Tanner stage Ph1, B1. Bone age was slightly advanced.

Serum calcium, phosphorus, PTH, calcitonin were in the normal range. Metabolic screening was normal. Karyotype: 46,XX. Molecular studies for Prader-Willi, Angelman syndrome and matUPD14 were carried out with normal results. Fundus Oculi: light pale optic discs, with shading edges. Neuropsychiatric evaluation showed severe mental retardation. EEG and cerebral MRI were normal.

We propose the diagnosis of MOMO syndrome (Macrosomia-Obesity-Macrocephaly-Ocular abnormalities), even if no major ocular defects (i.e. coloboma, glaucoma) have been found in the proposita. Alternatively a new syndrome could be suggested.

P0166. Decreased mineral bone density: Previously unrecognized feature of Fabry disease

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Background: Fabry disease (FD, OMIM 301500) is an X-linked inborn error of metabolism due to the deficient activity of alpha-galactosidase, a lysosomal enzyme. While the progressive deposition of glycosphingolipids throughout the body is known to have protean clinical manifestations, no data is available regarding the skeletal involvement.

Methods: We prospectively investigated bone involvement in 31 consecutive hemizygous males with a mean age of 32 years (median 28 years, range 16-61 years) affected with classic FD. Bone densitometry of the lumbar spine and femoral neck was assessed in all patients, using dual energy X-ray absorptiometry.

Results: Bone densitometry examination revealed a statistically significant decrease in bone mineral density. Using the World Health Organisation (WHO) classification of bone mineral density (BMD) abnormalities, the following results (N=31) were obtained at testing lumbar spine and femoral neck (Table).

Twenty-four patients had normal kidney function.

Discussion : This is the first study demonstrating that FD is associated to an increased risk of developing bone mineral density abnormalities

in male patients. Osteopenia and/or osteoporosis were detected in 84% (26/31) of our patients, even in the absence of renal insufficiency. We advise to perform bone densitometry in all FD patients to assess their risk of pathologic fractures. With emerging enzyme replacement therapies for FD and the hope of increased life expectancy, reduced bone mineral density may become an important symptom to consider.

WHO BMD Class	Number of patients (%)
Normal	5 (16%)
Osteopenia at 1 site, normal value at other site	10 (33%)
Osteopenia both sites	6 (19%)
Osteoporosis at 1 site, normal value at other site	1 (3%)
Osteoporosis at 1 site, osteopenia at other site	8 (26%)
Osteoporosis both sites	1 (3%)
Severe osteoporosis	0

P0167. An atypical swelling of the forearm

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Here we report on a 3 years old Tunisian girl, born from consanguineous parents, who was referred to the pediatrician because of a congenital swelling of her right forearm just below the elbow joint. A biopsy of the swelling was taken and histopathologic examination revealed that the lesion matched the criteria for a Gardner-associated fibroma (GAF) which can be a feature of Gardner syndrome (GS).

Gardner syndrome (MIM#175100) is characterized by intestinal polyposis and various bone and soft-tissue lesions, including e.g. osteomas, fibromas, and desmoid fibromas. It is considered a variant of familial adenomatous polyposis coli (FAP). GS patients inevitably develop intestinal carcinoma at a much younger age than those with sporadic intestinal carcinoma. Typically, the soft-tissue lesions occur before the development of intestinal polyps. GS is caused by truncating mutations in a portion of the APC-gene on chromosome 5q22-23 (codons 1403 and 1578) that differs from classic FAP (codons 169-1600).

In our proband a heterozygous truncating mutation in codon 1465 (exon 15); 4393_4394delAG (Ser1465fs) was found. DNA analysis in her parents and paternal half-sister did not show the mutation.

Accurate histologic identification of a GAF, even in an unusual place like our proband's forearm, represents a sentinel event for the detection of unsuspected GS. It allows for early and close monitoring of GS symptoms and for possible preventive interventions.

P0168. Diastrophic dysplasia - early detection of a new case

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Diastrophic dysplasia (Maroteaux-Lamy syndrome) is a very rare form of chondrodysplasia, recessively inherited, characterized by prenatal dwarfism, limb shortening, abnormal ears, progressive joint deformities and contractures, typical hand defect ("hitchhiker thumb"), progressive spinal curvature and clubfeet. We present a new case in order to illustrate this rare entity and to discuss the diagnosis and the management. Our proband is a one-month-old male infant, the third child of a young, unrelated, apparently normal couple. The pregnancy was uneventful and no fetal ultrasound scan was done. He was born at 32 weeks gestation (Wt 1650 g, Ht 40 cm, HC 31 cm, Apgar score 6). A suspicion of diastrophic dysplasia was established after birth. The infant was reexamined one month later, when physical examination revealed: short limbed dwarfism, dysmorphic face (mild exophthalmia,

cleft soft palate, microretrognathia and low-set, thickened ears), short neck, thumbs and halluxes in abducted position bilaterally. Radiological examination showed typical aspects. We have established the diagnosis of diastrophic dysplasia based on the characteristic association of short limbed dwarfism, cleft palate, thickened ears and "hitchhiker thumb". Differential diagnosis was done with other types of short limbed dwarfism (camptomelic dysplasia, Ellis van Creveld etc). The plan for the management and the follow up of the patient will be presented.

In conclusion, we present a new case of diastrophic dysplasia in order to illustrate this rare genetic disorder, but also to discuss the importance of a complex medical specialist team for a correct diagnosis and management of the affected family.

P0169. A particular case of ring chromosome 5

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Ring chromosome 5 is a rare chromosomal abnormality expressed clinically by features of cri-du-chat syndrome associated with other features depending on the fragment lacking from the long arm. We present a particular case of r5 chromosome in order to illustrate this rare entity and to discuss diagnosis and management. Our proband is a 2 years 5 months old female child, single child of a young, unrelated, apparently normal couple. The pregnancy evolved with polyhydramnios. No foetal ultrasound scan was performed. She was born at 9 months gestation (Wt 1550 g, Ht 38 cm, HC 28 cm, Apgar score 8) and needed intensive care. Postnatal development was severely delayed. Physical examination revealed: proportionate dwarfism (Ht -2.86 SD, Wt -4.66 SD) associated with microcephaly (HC -5.08 SD), dysmorphic face (triangular face, hypertelorism, abnormal, asymmetric ears), mewing cry and severe developmental delay. Echocardiography: subaortic VSD. Renal echography: normal. Psychological examination: IQ 45. We thought of Cri-du-chat syndrome reason why a karyotype was indicated. The result was: 46,XX,r5/ 45,XX,-5/ 47,XX,r5,r5. Differential diagnosis was done mainly with the typical form of cri-du-chat syndrome. The plan for the management and the follow up of the patient will be presented, as well as the hypothesis for the mechanism that led to this disorder.

In conclusion, we present a particular case of ring 5 chromosome in order to illustrate this rare genetic disorder, but also to discuss the importance of different features for the diagnosis and the management of the family.

P0170. False-positive proportion for pediatric ammonia determination and its clinical impact

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Objective: Plasma ammonia measurement is critical for the diagnosis and management of several inborn errors of metabolism. Its determination is subject to several sources of error. False-positive results might be harmful to patients. We wanted to evaluate the prevalence and clinical impact of false-positive results for plasma ammonia in a pediatric tertiary care hospital.

Design: Data from the clinical biochemistry laboratory were scrutinized over a 28-month period. Each case that presented at least one abnormal result was revised. These files were reviewed to determine if a medical condition could account for the initial elevation of ammonia and its subsequent normalization.

Results: 1980 ammonia measurements were obtained from 479 patients. Ammonemia concentration ranged from 5 to 1863 µmol/L. Median values for neonates (less than 1 month) and older patients were 65 and 50 µmol/L respectively ($p<0.001$). Elevated results were found in 42% blood samples from 151 patients. Among 86 files revised, we have found 41 false-positives for plasma ammonia, which represents 48%. Capillary samples were overrepresented among false-positives. There was also a long delay of 7,9 hours between the falsely elevated value and its subsequent normal value for hospitalized patients. The major clinical impact was regarding additional laboratory analyses. There were no prolonged hospitalization attributable to a false-positive.

Conclusion : This study shows the high proportion of false-positive regarding ammonia measurement in pediatric population. Fortunately, the clinical impact seems to be limited but not negligible, especially for patients with inborn errors of metabolism. Capillary sample should be avoided for ammonia determination.

P0171. Clinical features in patients with mutations in JARID1C

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We have recently shown that mutations in JARID1C are one of the more common known causes of X-linked mental retardation (XLMR) (see abstract of Jensen et al. at this conference). Here, we report on the clinical phenotypes of 21 patients with JARID1C mutations. Mental retardation of various severity is the prominent clinical feature in all patients. In addition, short stature is also present in the majority of cases. Numerous dysmorphic signs and neurological features have been observed in some but not all patients. These include microcephaly, maxillary hypoplasia, cryptorchidism, macroorchidism, spasticity and epilepsy. We will give a detailed account of the individual phenotypes of the patients with JARID1C mutations. This will help to identify patients in whom mutation screening in JARID1C should be considered.

P0172. Mutational analysis of the 21-hydroxylase gene, CYP21, in 17 Estonian Patients Diagnosed with Congenital Adrenal Hyperplasia

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At least 90% of all cases of the autosomal recessive condition Congenital Adrenal Hyperplasia (CAH) are caused by mutations in the 21-hydroxylase gene (CYP21). The most common mutations found in CYP21 are a result of either unequal crossing-over or gene conversion events involving the neighbouring, highly homologous pseudogene, CYP21P, on chromosome 6p21.3. We have screened 17 Estonian patients (in most cases with both parents), clinically diagnosed with CAH, for eight common CYP21 mutations using a series of PCR ARMS tests. 18 % (6/34) of all CYP21 alleles harboured a chimeric pseudogene:gene mutation consisting of 5' CYP21P sequence and 3' CYP21 sequence and arising from a large scale deletion event. A c.292 -13A/C>G (g.655 A/C>G) intron 2 splice site mutation, which is found at a similar frequency to CYP21 deletions/conversions in the UK population, accounted for only 6% (2/34) of alleles. The point mutations Ile172Asn, Gln318Stop, and Arg356Trp all occurred at a frequency of 9% (3/34) while one patient harboured four sequential point mutations suggesting a large conversion of the 3'end of CYP21 to CYP21P. Work is ongoing to identify further CYP21 mutations in remaining alleles by Southern blot and sequencing analysis.

P0173. A novel KCNQ2 splicing mutation causes BFNC in a large multi-generational family

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Benign familial neonatal convulsions (BFNC) is a rare epilepsy disorder with autosomal dominant inheritance and high penetrance. Two

genes have been identified to be responsible for BFNC, KCNQ2 at 20q13.3 and KCNQ3 at 8q24. KCNQ2 and KCNQ3 encode potassium channel subunits that mediate the M-current, which limits neuronal hyperexcitability through spike-frequency adaptation. The study of new pedigrees may help to detect new mutations and define genotype-phenotype correlations, leading towards a better understanding of the pathophysiologic mechanisms underlying neonatal convulsions. We revisited a BFNC family with 13 affected members in five generations in order to assess long-term outcome after 20 years of follow-up: 11 showed remission within two months with normal neurological development, one had mild learning disabilities and one developed photosensitive myoclonic epilepsy later in life. Molecular genetic analysis revealed linkage to chromosome 20 and a single intronic mutation IVS14-6 C>A in KCNQ2 that segregated with the BFNC trait in all affected members, and was absent in 100 unrelated controls. Taking advantage of the fact that tissue-specific genes may be expressed ubiquitously at very low levels (illegitimate transcription), we evaluated the effect of the KCNQ2 mutation at the mRNA level using reverse transcribed total RNA isolated from blood leukocytes. This mutation creates a new splice site preferentially used for splicing. Alternative splicing adds 4 nt containing a premature stop codon to the transcript, which results in a truncated protein after position R588. This novel KCNQ2 mutation cosegregates with BFNC but not with photosensitivity.

P0174. Clinic and genetic heterogeneity in Ehlers-Danlos syndrome

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Ehlers-Danlos syndrome (EDS) is a heterogeneous group of heritable connective tissue disorders characterized by articular hypermobility, skin hyperextensibility, and tissue fragility. EDS type IV being the most life-threatening form. It is characterized by a type III collagen deficiency and this disease involves a *col3A1* gene mutation. We report the case of a 47 year-old woman with type IV EDS. The medical history of our patient included multiple spontaneous bone fractures, anomalies of subclavian artery, moderate bruising and rupture of hollow organs such as the intestine and stomach, requiring repeated surgical interventions, generalized joint hypermobility, skin hyperextensibility, chronic joint pain, recurrent joint dislocations, extensive bruising, characteristic facial appearance, varicose veins, progressive scoliosis, osteopenia. Each of her two children presented clinical elements of EDS: her daughter (25 years old) presented especially molluscoid pseudotumours, subcutaneous spheroids joint hypermobility, chronic joint pain with recurrent joint dislocations, easy bruising and spontaneous bone fractures. Her son (18 years old) presented recurrent joint dislocations, moderate skin hyperextensibility, articular hypermobility and autism. Clinic and genetic heterogeneity of the disease is very evident in this family, the three family members presenting clinical symptoms and comorbidities which made difficult the attempt to integrate them in a certain EDS type; these three cases presented a various clinical expression and severity. Another particularity is also represented by the presence and high frequency of associated spontaneous bone fractures. Molecular investigations could probably explain the mechanism which associates Osteogenesis Imperfecta signs to EDS symptoms, but we couldn't perform these investigations for the time.

P0175. Adolescental problems in KBG syndrome: our experience

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KBG syndrome is a rare, but underestimated, autosomal dominant disease (40 cases described), characterized by macro-oligodontia, short stature, mild to medium mental retardation, skeletal defects and other clinical features. We report 7 sporadic cases, 3 M and 4 F, observed during adolescence. This period is characterized by the modification of previous relationships, that leads, to a possible change in the role and importance of the family and reference figures. The child grows and "the chrysalis becomes butterfly". The process seems complicated and difficult in KBG patient. He links superficially to other persons. His world remains limited to a biunivocal relation with the mother, that lasts longer than the other children, so remaining in a

condition of passivity and greatest dependence. He understands he doesn't overtake the daily problems without help. He reacts isolating from the real life, taking refuge in a fantastic world. He closes himself in a autistic passivity as the only answer to his own inadequacy. The world outside seems to him full of hostility.

More than ten years ago we chose, in favour of patients with genetic syndromes, a multidimensional and multidisciplinary approach coordinated by a clinical genetist. Therapeutic results have been satisfactory in KBG patients.

We noted: acceptance of their own body, more optimistic approach to life, improvement of school and working integration, higher control of nutritional and drug addiction.

This experience improved our life and our profession, allowing a deep and empathic interaction between patient and physician, that represents the true essence of every medical act.

P0176. A quantitative definition of monosomy 5p syndrome.

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Monosomy 5p syndrome (cri du chat)(OMIM#123450) belongs to a group of classical chromosomal entities with characteristic facial traits, organ malformations, functional impairment and developmental delay due to a partial monosomy of a short arm of chromosome 5. Although several hundreds of cases have been published to date, a systematic collection of its clinical symptoms and anthropological traits is missing in the literature. Today, as cases with sub-microscopic 5p deletions are known, and CDCS genes are being searched, more exact knowledge seems to be necessary. The main aim of this work is a contribution for quantitative syndrome definition obtained by systematic evaluation of clinical symptoms and anthropological traits. A group of 22 children with terminal 5p deletion aged between 1 month and 18 years were examined. A catalogue of well-defined 807 dysmorphic and clinical features from the Munich Dysmorphology Database according to Stengel-Rutkowski was used. Anamnestic data were obtained from the parents and hospital records. A semi-standardized 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. The trait list for each child was set up by checking all informative features for presence or absence. Seventy two clinical and anthropological features have been observed with a frequency of 50% or more (at least 5 informative features) in our group with monosomy 5p. On this basis quantitative phenotype definition of monosomy 5p syndrome was suggested.

P0177. The first case of galactosialidosis (Goldberg syndrome) in the St.Petersburg population

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Galactosialidosis (GSL, Goldberg syndrome, MIM 256540) is a lysosomal storage disease with autosomal recessive type of inheritance, gene map locus 20q13.1. The condition is caused by a deficiency of carboxypeptidase-L protective protein, which forms a high molecular weight complex with alpha-neuraminidase and beta-galactosidase in the lysosomes. Proband, a male aged 1 year, 3 months and 25 days, is under our observation. He was born after the second spontaneous premature delivery. This pregnancy was complicated with late toxemia and threatened abortion. His parents are nonconsanguineous, his elder brother is healthy. Proband birth weight was 2850g. At birth severe oedema of eyelids, face and scrotum, moderate hepatosplenomegaly, hyperbilirubinemia were observed. At age 1 year and 4 months the proband had some additional symptoms of dangerous illness:

coarse facial features, mental retardation, telangiectasia of skin, increased hepatosplenomegaly, hypotonia, some biochemical signs of metabolic disturbance. Oligosacchariduria and beta-galactosidase and neuraminidase deficiency were shown in the Scientific Centre of Medical Genetics, Moscow. These data confirmed our presumed diagnosis of galactosialidosis, early infantile type.

P0178. Collecting oro-dental phenotypic data: standardisation and networking via D[4]/ PHENODENT

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The aims and objectives of the D[4]/ PHENODENT project are to create a collaborative interactive biomedical database D[4] (Diagnosing Dental Defects Database) linked to a dynamic web site PHENODENT allowing standardisation of data collection and therefore assisting in oro-dental phenotyping.

This tool will permit integration of these data within the medical and genetic general context enhancing multidisciplinary patient management approaches. It will facilitate understanding of dental and oral biology and associated disorders and diseases implementing science based evidence diagnosis and therapeutic options. These data might be used in public health as markers of gene/environment relationships in the case of acquired dental defects. D[4]/ PHENODENT will stimulate patient recruitment and install a basis for molecular analysis and anatomopathological investigations. It will allow the creation of larger cohorts of patients, presenting with these rare oro-dental defects, that could be involved in future research projects like - Oro-dental phenotypes in syndromes - Identification of mutations in known genes involved in dental development and diseases - Phenotype/genotype correlation - Population genetics - New gene identification - Gene expression during odontogenesis - Mouse/Human correlations. Standardisation will facilitate sharing of data and materials among investigators.

PHENODENT will constitute a link between participating clinical diagnosis centres and research laboratories thus representing a powerful tool for national (French INSERM GIS rare diseases, Odontogenetics network) and international (European COST) networks.

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P0179. Detoxification system genes involved into the resistance to the steroid therapy in asthmatic patients.

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Steroids, using for the reducing of asthma symptoms in patient with severe disease was shown as non-effective in 34% of patients in St. Petersburg, Russia. The major role in inactivation almost all drugs, coming to the organisms from the environment, as well as steroid medications, play the detoxification system enzymes. Mutations in the genes coding this enzymes lead to the loss of function of complete absence of them. Thus, asthmatic patients, having detoxification genes deficiency, suppose to be very sensitive to the medications, using for asthma therapy. In the present study the distribution of normal and mutant genotypes Phase I detoxification gene CYP1A1 and Phase II detoxification genes NAT2, GSTT1 and GSTP1 did not show significant differences in patients with positive and negative response to the therapy. At the same time, we found strong association between GSTM1 gene deficiency in asthmatics and their steroid resistance: 93.3% of resistant patients had GSTM1 0/0 genotype. In the group of asthmatics giving the best response to the steroid treatment predominated individuals with normal genotype GSTM1 +/+, GSTP1 A/A : 20% vs 4.5% in the group of the drug resistant patients, p=0.01 (in the control group from the general population of St.Petersburg distribution of this genotype comprise 34.4%, p=0.0001).

Thus, Phase II detoxification enzymes coding by GSTM1 and GSTP1 genes are important for the steroid resistance of asthmatic patients.

P0180. Segmental Neurofibromatosis type-1 presenting as isolated plexiform neurofibromas

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We present a 41-year-old woman with recurrent plexiform neurofibromas without signs of classical Neurofibromatosis type-1 (NF-1). She presented with a superficial plexiform neurofibroma on the left scapula in adolescence. She was subsequently found to have a thoracic intraspinal neurofibroma, two plexiform neurofibromas in her left kidney and three plexiform neurofibromas in the subcutaneous tissue of her left posterior chest wall. Our patient underwent surgical debulking of her tumors but the plexiform neurofibroma in her chest wall persists. Physical examination reveals no café-au-lait macules, inguinal or axillary freckling, or Lisch nodules. There is no family history of NF-1 and her daughter has no clinical features of this disorder. There are a handful of Segmental NF-1 cases describing the occurrence of localized deep-tissue plexiform neurofibromas and no cutaneous manifestations. Local recurrence is common but malignant transformation of the plexiform neurofibromas has not been reported in these cases. It is felt that this rare form of Segmental NF-1 is due to a post-zygotic mutation in the neurofibromin gene in early development. Subsequent loss of heterozygosity in this cell line may account for the development of the plexiform neurofibromas. Molecular investigations have been initiated in our patient to further characterize these tumors. We feel that this condition should be distinguished from generalized NF-1 and other similar disorders for the purposes of medical management and recurrence-risk counseling. Screening of individuals who present with isolated plexiform neurofibromas for other tumors should be considered as they may have Segmental NF-1 and be at risk of developing further neurofibromas.

P0181. Cornelia-de-Lange Syndrome, Phenotypic and Cytogenetic Analysis of 20 Iranian Cases; Clinical Case Report

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Cornelia-de-Lange syndrome (CDLS) is a rare multisystemic malformative syndrome of uncertain etiology characterized by severe psychomotor and mental retardation, and multiple congenital anomalies.

Here we report the phenotypic and cytogenetic analysis of 20 cases of Iranian patients who displayed the classical clinical symptomatology of CDLS.

In this report, we have done necessary evaluation of clinical findings, chromosomal studies and laboratory investigations.

Clinical manifestations in our patients were as similar as the cases were reported in the medical literature. Chromosomal analysis by traditional GTG banding technique; in all of the affected patients were normal. We did not do FISH analysis in our cases. The diagnosis of CDLS in this patients were based on clinical evaluation and comparison of the findings with reported cases by Brachmann in 1916 and Cornelia-de Lange in 1933 and other reported cases.

P0182. Severe zinc deficiency in Thalassemia Major patients on deferiprone

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Thalassemia major demands frequent blood transfusion to maintain life while hemosiderosis requires continuous iron chelation. Increased zinc excretion is known to occur in thalassemic patients receiving regular chelation therapy with deferiprone. This study was conducted, firstly, to examine serum zinc level in thalassemic patients in order to know how much zinc supplementation was required and secondly, to see if zinc supplementation contributed towards better growth. Methods: Study was conducted on 86 thalassemia major patients on hypertransfusion treatment aged between 4 and 19 years at SGPGIMS. Zinc levels were checked before and after zinc supplementation. Low dose of zinc 23.5 mg as Zevit capsule was given once a day for one year. Growth velocities and pubertal development were evaluated every 3 months. Results: Out of 86 patients 52 (60.4%) had low serum zinc level. Compliance to zinc supplementation was significantly associated

with increased serum zinc levels. Pre supplementation mean height and weight velocities were 4.54 (\pm 2.26) and 1.4 (\pm 1.46) and post supplementation were 4.71 (\pm 2.38)cm and 1.7 (\pm 1.02) respectively. In individual cases post supplementation height and weight were higher compared to pre supplementation. Serum ferritin was negatively correlated ($P<.001$) with pre and post supplementation growth velocities. Pre and post supplementation serum ferritin and hemoglobin levels were strongly correlated ($P<.001$). Compliance to zinc supplementation was negatively correlated with pre-supplementation hemoglobin levels. Conclusion: With optimally maintained hemoglobin level zinc supplementation given in high doses can have beneficial effects on growth.

P0183. GM1 gangliosidosis: Clinical and laboratory findings in one Iranian family with 5 affected patients.

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GM1 gangliosidosis is an autosomal recessive genetic disorder due to deficiency of lysosomal enzyme beta-galactosidase, with the consequence of tissue accumulation of especially GM1 ganglioside. In the present paper, we report the clinical and laboratory findings of an Iranian family that have had history of five affected patients. Four of them died in about four to five years of age, because of probable diagnosis of Niemann-Pick disease. Parents are first cousin relatives there were no similar cases in other close relatives. The family have only one healthy daughter, she is 11-year-old and product of 8th pregnancy of mother. Our proband is also suffering from the same clinical feature of deceased siblings. She was about 4-year-old when referred to us. Hypotonia, neuromotor retardation, hepatosplenomegaly, and coarse facies were the cardinal clinical findings. The disease evolves towards convulsions and blindness, deafness, and ultimately decerebrate state. Leading to patient's death when she was five years old. After complete clinical and laboratory investigation, we found abnormal foamy histiocyte in the bone marrow aspirate. Then we sent blood and skin biopsy samples to the Metabolic Service of Erasmus University in the Netherlands. Because of complete deficiency of enzyme beta-galactosidase in her WBC, and skin fibroblasts, final diagnosis in this family was **GM1 gangliosidosis**. This is the first diagnosed family confirmed by enzyme assay from Iran.

P0184. Myotonic dystrophy in the Republic of Sakha (Yakutia) (Russia)

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During last 20 years 105 patients with diagnosis myotonic dystrophy (MD) from 39 families are registered. Now 98 is alive (58,16 % - women, 41,84 % - men). 92 of them are Yakuts (93,88 %) from 35 families. The prevalence of MD in the Yakut population (total population is about 420 000) is 23,28 on 100 000. Disease is registered in 18 of 35 administrative units. Prevalence of MD in separate areas changes from 2,23 up to 122,22 on 100 000 population. Among agricultural population where the indigenous population makes the majority, prevalence MD in 4,2 times is higher, than among city.

In regional medical genetic consulting department there are 81 patients with clinical diagnosis "myotonic dystrophy" in the age of from 0 till 63 years and 173 their clinically healthy relatives. Among affected patients usually occur classical adult form of MD (45,7%), in 2,5% - mild form, in 35, 8% - juvenile and congenital form - 3, 7%. Molecular-genetic diagnostics is carried out by method PCR with the subsequent electrophoresis. There were 9 prenatal diagnostic investigations in 8 affected families.

Probably, one of the reasons of accumulation MD among indigenous population is the effect of the founder. The further researches are necessary for an explanation of the reasons of high prevalence MD in Yakuts, expansion of opportunities of medical-genetic consultation of families with MD with use of molecular-genetic methods with a view of reduction of a genetic cargo.

P0185. Autosomal dominant inheritance of cardiac valve anomalies

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Anomalies of the atrioventricular and semilunar heart valves account for 25 -30 % of all congenital heart malformations (CHM). The majority of these heart malformations are sporadic, although some pedigrees with autosomal dominant inheritance of non-syndromic heart valves anomalies have been described. Cardiac valve anomalies can also be part of well-defined syndromes with autosomal dominant inheritance (such as Noonan syndrome and Alagille syndrome).

We describe here two multiplex pedigrees with non-syndromic forms of heart valve anomalies that segregate as an autosomal dominant condition.

The first family is a 3-generation pedigree with 9 family members affected with congenital defects of the cardiac valves, including aortic stenosis (4 patients), aortic (1 patient) and mitral (3 patients) valve insufficiency, and bicuspid aortic valve (2 patients). Pulmonary atresia, pulmonary stenosis and tricuspid insufficiency was present in one patient each. Several patients had septal defects. The second family consists of 7 patients in 2 generations with valvular aortic stenosis in 3 patients. Two patients presented with defects of the pulmonary valves; one with pulmonary atresia and narrow tricuspid valve and a second with valvular and infundibular pulmonary stenosis with intact ventricular septum. Both patients also had an atrial septal defect. These families might be instrumental in identifying genes involved in cardiac valve formation and malformation.

P0186. Michels Syndrome, Carnevale Syndrome, and Malpuech syndrome: Variable Expression of a Single Disorder (M2C syndrome) ?

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We report a 3-year-old girl with Michels syndrome, a rare condition characterized by craniosynostosis, blepharophimosis, ptosis, epicanthus inversus, cleft lip/palate, abnormal supra-umbilical abdominal wall and mental deficiency. The phenotypic findings are compared with the 6 previously reported Michels cases, and with patients referred to as Carnevale, OSA and Malpuech syndromes. Michels syndrome is characterized by cleft lip and palate, anterior chamber anomalies, blepharophimosis, epicanthus inversus, and craniosynostosis. Carnevale syndrome shows hypertelorism, downslanting palpebral fissures, ptosis, strabismus synophrys, large and fleshy ears, and lozenge-shaped diastasis around the umbilicus. OSA syndrome resembles Carnevale, with humeroradial synostoses and spinal anomalies as extra features. Malpuech syndrome shows IUGR, hypertelorism, cleft lip and palate, micropenis, hypospadias, renal anomalies and caudal appendage. All are autosomal recessive. Despite the presence of distinctive key features, it appears that these 4 entities share multiple similarities in the facial Gestalt and the pattern of MCA. Those similarities lead us to postulate that they could belong to the same spectrum, which could be referred to as "M2C syndrome" (Malpuech-Michels-Carnevale syndrome).

P0187. Familial Rubinstein-Taybi syndrome

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Rubinstein-Taybi syndrome (RTS) is a clinically well-defined syndrome characterized by a combination of broad thumbs and halluces, characteristic facial dysmorphism, growth retardation, and mental deficiency. RTS was shown to be associated with disruption of one copy of the CREB-binding protein gene, either by gross chromosomal

rearrangements or by point mutations. It was supposed that RTS has usually sporadic occurrence, but there have been few reports of familial cases with autosomal-dominant inheritance. We report a new familial occurrence of RTS in two generations (a boy, his father and fathers' sister).

Patient 1, 8-year-old boy has short stature (-2,5 SD), highly arched eyebrows, down-slanting palpebral fissures, ptosis, epicanthal folds, short convex nose, long columella, long philtrum, low-set dysplastic ears, broad thumbs and halluces, clinodactyly of fifth finger and unilateral cryptorchid testis. Ultrasound investigation showed horseshoe kidney. The speech disturbance was diagnosed. The father of patient 1 has only broad thumbs and halluces, but normal growth and development.

Patient 2, 48-year-old aunt of patient 1, who also has short stature (-3,5 SD), obesity (+4,5 SD), normal mental development and similar facial features - down-slanting palpebral fissures, short prominent nose, long columella, high palate, low posterior hairline, broad thumbs and halluces.

At least 7 familial cases of RTS or Rubinstein-like syndrome have been described. Most of the patients in familial cases are mildly affected and with heterogeneous phenotype like in our described family. This family supports the conclusion of Bartsch et al. (2002) that mild alleles or modifying factors can lead to incomplete RTS.

P0188. Gene polymorphisms COL1A1 and VDR analysis in patients with syringomyelia from Bashkortostan.

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Syringomyelia is a neurodegenerative disorder, resulting from spinal cavity formation. To study the role of genes involved in the functioning of connective tissue, we performed Snp polymorphism analysis in the *COL1A1* gene (collagen, type I, alpha-1, 17q21.3-2) and Fok-I RLPA analysis in the *VDR* gene (vitamin D receptor). The groups analyzed included 116 patients with syringomyelia and 185 healthy individuals from 3 ethnic groups: Russians, Tatars, Bashkirs.

The Snp allele frequency distribution showed statistically significant differences between patients of Tatar and Russian origin. There were no statistically significant differences between Russians, Tatars and Bashkirs in the control group. There were also no statistically significant differences in allele and genotype frequencies at this locus in affected patients as a whole and in ethnically different groups of patients as well as in controls, although in patients and controls of Russian origin the frequency of the s allele, corresponding to less active form of syringomyelia was 0.258 in affected patients and 0.108 in controls (OR=2.863). This result lets us suggest that the s allele may be a risk factor for disease development.

In the *VDR* gene Fok-I polymorphism there were no significant differences in genotype frequencies between patients and controls. FF genotype is significantly more frequent in patients than in controls (P=0.0005, OR=3.71). There were no differences in allele frequencies between patients and controls in this locus.

So, according to our data, FF genotype may be considered as a susceptibility genetic marker for syringomyelia.

P0189. Preliminary results of hMLH1 and hMSH2 mutation analysis among Iranian patients with HNPCC

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Background:

Hereditary non-polyposis colorectal cancer (HNPCC) is one of the most common inherited cancer syndromes, accounting for 3-5% of all cases of colorectal cancer.

Germline mutations in the DNA mismatch repair (MMR) genes MSH2 and MLH1 are responsible for the majority of HNPCC patients

Methods:

We screened 41 Iranian patients who fulfilled the Bethesda criteria for HNPCC. Germline mutations for hMLH1 or hMSH2 genes were evaluated in all 41 patients. Mutation analysis was performed by PCR amplification of the 19 exons for hMLH1 and 16 exons of the

hMSH2 genes. The amplicons were screened by SSCP for mutations. Samples which showed band shifts on the SSCP were verified by direct sequencing.

Results:

For the hMLH1 gene we found one missense mutation in the exon 11 at c.326T>C(Val - - Ala) and two nonsense mutations in the exons 13 and 19 at c.487C>T(Arg - - Stop) and c.2146_2147 delGT(Val - - Stop), respectively.

For the hMSH2 gene an insertion was found at the nucleotide 3010 Ins G causing a frameshift in the exon 13. This mutation was found in four patients. In one of the patients another transversion was also found at c.677C>G(Thr - - Arg) in the exon 13 besides the 3010InsG.

Discussion:

This is the first report of the mutational analysis of patients with HNPCC in Iran. Our results provide further insight into the mutational spectrum of MMR genes in HNPCC families.

P0190. Genotype-phenotype correlations of the Wilson disease in Volga-Ural region.

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Wilson's disease is an autosomal recessive disorder of hepatic copper metabolism caused by mutations in the gene encoding a copper-transporting P-type ATPase (13 q 14.3-q 21.1) and leading to hepatic or neurological disease.

Wilson disease has an irregular ethnic and geographic occurrence in populations and is characterized by interfamily and intrafamily variability.

The purpose of this research was the analysis of the correlation between clinical features (neurological, neuropsychological and liver disorders) and types of mutations of 29 patients from Bashkortostan. Using SSCP analysis followed by sequencing of shifted exons we have identified 4 mutations: His1069Gln (43.5%), 3402delC(6.5%), 3559+1G>T (6.5%), the Glu1064Lys substitution was found to be heterozygous in two WD families.

All patients were divided into 3 groups: 1-homozygous for the His1069Gln mutation, 2-compound-heterozygous for the His1069Gln mutation and 3-patients with unknown mutations.

Our results show that homozygotes for the His1069Gln mutation had a milder disease onset at a later age and rare severe liver disorder.

In several cases we found an interfamily variability between sibs in age of manifestation and character of clinical symptoms, which could not be explained by the types of mutation.

According to our studies Wilson disease is more frequently in the Tatar population and has a severe disease course.

Our results indicate that Wilson disease has interfamily and intrafamily variability which needs to be studying further.

P0191. Sotos syndrome. Novel and known mutations of NSD1 gene, in a new group of unpublished Italian patients.

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Sotos syndrome (OMIM #117550) is an overgrowth syndrome characterized by pre- and post-natal overgrowth, macrocephaly, advanced bone age, mental retardation, and typical facial features. The recent identification of deletions and point mutations of NSD1 (Nuclear receptor-binding SET domain containing protein) in more than half (> 60%) of Sotos syndrome subjects has allowed the re-evaluation of clinical phenotype and natural history of this disorder for a genotype/phenotype correlation. In the same time, the interest is growing to identify other molecular defects underlying about 40% of Sotos syndrome typical cases.

We analyzed genomic DNA from 12 patients with Sotos syndrome (isolated and familial cases) for mutations of the NSD1 gene, by using direct sequencing and MLPA analysis. All patients were included in the register of our Unit with the diagnosis of Sotos syndrome, following an accurate clinical evaluation and a regular follow-up specific programme, performed in the same Genetics Unit since many years. In all cases,

G and Q banding chromosome analysis, from phytohaemagglutinin stimulated peripheral blood lymphocyte cultures using standard procedures, showed a normal karyotype

Based on the published genomic sequence, for the direct sequencing analysis, 40 primer pairs were used for PCR amplification of exons and splicing junctions of the NSD1 gene. For the MLPA technique, we used the probemix P026B for 24 NSD1 gene regions. We were able to confirm the clear predominance of point mutations, with the identification of well known, or not yet described exonic mutations in the coding regions, and intronic mutation at the splicing sites.

P0192. Muenke craniosynostosis: mutational analysis of FGFR3 gene improves the identification of an otherwise clinically undiagnosed disorder

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Proline to Arginine gain-of-function mutation in FGFR3 gene (Pro250Arg), caused by a C749G transversion, results in Muenke syndrome (MS), characterized by coronal precocious synostosis, macrocephaly and abnormal skull shape. A high arched palate, sensorineurial hearing loss, and developmental delay can also be evident. The FGFR3 gene maps to human chromosome 4p16.3: consists of 19 exons and 18 introns. FGFR (Fibroblast growth factor receptor) 3 gene, is associated with other genetic disorders (Achondroplasia, Hypochondroplasia, SADDAN syndrome, Lethal Thanatophoric Dysplasia, some cases of Crouzon syndrome) with different, recurrent, mutations.

We report the preliminary results of a two years project designed to identify the Muenke syndrome, associated with the FGFR3 gene mutations in newborns and childrens with apparently isolated coronal craniosynostosis, or with a syndromic phenotype suggestive for Muenke syndrome.

The direct sequencing analysis revealed the same Pro250Arg mutation in seven patients. One family with almost three affected subjects. A couple of MZ twins, and two other unrelated subjects with a *de novo* mutation. We emphasize the variable clinical phenotype, also in the same family: in particular, with complete or partial, bicoronal or unicoronal synostosis. In the MZ twins, craniosynostosis was identified during the pregnancy by morphological fetal study, early in the third trimester.

P0193. High frequency of skewed X chromosome inactivation in Rett patients and their mothers

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Rett syndrome is an X-linked neurological disorder with a characteristic phenotype, and substantial phenotypic variability. This variability has been partly attributed to an effect of X chromosome inactivation. A higher frequency of skewed X inactivation in Rett patients has been reported. We report here a study of X inactivation ratios in DNA from blood and buccal cells of Rett patients (N = 69) and their mothers (N = 73). The mean degree of skewing in blood was higher in patients (71.1%) than controls (65.7%) (N = 58; p<0.01). Unexpectedly, the mean degree of skewing in blood was also higher in the mothers (71.5%) (p<0.01). The Rett patients and the mothers had a higher frequency (25 % and 29 %) of skewed X inactivation (defined as >80 %) than controls (16 %), but the difference was not statistical significant. The mean degree of skewing in buccal smear was lower than for blood, and was significantly higher in mothers than in controls (p<0.05). To test whether the Rett patients with skewed X inactivation were daughters of skewed mothers, 53 mother-daughter pairs were analysed. There was no evidence for a relationship between skewing in patients and mothers; of 14 patients with skewed X inactivation, only 3 had a mother with skewed X inactivation. We conclude that Rett patients and their mothers may have a significantly greater degree of X inactivation skewing than controls, and suggest that this phenomenon

may be of interest in the analysis of genotype-phenotype correlations in Rett syndrome.

P0194. Oral clefts: associations with epidemiological and clinical characteristics among newborns in Lithuania, 1993 - 1997

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The clefts of lip, alveolar ridge and palate are common heterogeneous congenital anomalies. Epidemiological studies of oral clefts in various geographical and ethnical areas exhibit some differences. The incidence of oral clefts nowadays is not falling and sometimes shows a tendency to an increase. The aim of the study was to establish the incidence rate of oral clefts in Lithuania and in its separate regions during 1993-1997; to create structural model registry and to perform a retrospective comparative analysis of oral clefts.

The incidence rate of oral clefts for 1000 livebirths in Lithuania during 1993-1997 was 1.84 (1:544), and in separate regions of Lithuania the rate of clefts for 1000 livebirths was from 0.01 to 3.34. Isolated clefts formed 74.1% of all clefts. Syndromic clefts formed 25.9% of all clefts and comparing to the period 1953-1964 showed a statistically reliable increase owing to improved diagnostics and registration. Unilateral clefts are more frequent than bilateral, and the left unilateral cleft is more common than the right. The most common diagnoses were isolated palatal clefts forming 40.4% of all clefts and statistically reliably diagnosed more for female patients. Unilateral total upper lip, maxillary alveolus and palate cleft was diagnosed for 21.8% of all clefts and was statistically reliably more common for male newborns. Different regions of Lithuania were divided into four clusters and the incidence of clefts cases per 1000 newborns in 1993-1997 did not differ statistically in the administrative regions of Lithuania.

P0195. Cytogenetics & Molecular Study of Acquired and Congenital Disorders

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The present study aimed to use fluorescent-labeled probes for screening various congenital anomalies (sex chromosomal and autosomal), prenatal diagnosis and cancer genetics. Standard techniques were used for conventional cytogenetics and Fluorescence In Situ Hybridization (FISH). Sex chromosome aneuploidies (XXY, XO, XXX, XYY) were analyzed using probes for chromosomes X and Y. The cases with ambiguous genitalia were further analyzed using probe for SRY gene. Prenatal diagnosis included screening aneuploidies of chromosomes 13, 18, 21, X and Y on uncultured cells and metaphases obtained from amniotic fluid and chorionic villi samplings. Gene alterations were studied in Retinoblastoma and leukemias using specific probes. Response to therapy was assessed by evaluating minimal residual disease (MRD) in leukemia patients. Attempts were also made to analyze cells obtained from buccal mucosa and bladder epithelium that could facilitate rapid screening anomalies without painful invasive techniques.

Prenatal diagnosis using FISH on uncultured cells provided accurate and rapid results within 24 hours. Molecular rearrangements that could not be detected with conventional cytogenetics were identified using FISH technique. The SRY region was detected in few cases of ambiguous genitalia that lacked the Y chromosome. Most leukemia patients in complete cytogenetic remission showed molecular evidence of MRD. Aneuploidies and gene rearrangements could also be detected on interphase (non-dividing) cells obtained from peripheral blood and bone marrow. To conclude, the present study stresses that FISH does add to the utility of cytogenetics by decreasing the time interval between sampling and diagnosis.

P0196. A patient with a *de novo* terminal deletion of chromosome 1q

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Terminal deletion of the long arm of chromosome 1 is characterized by multiple anomalies and neurological signs including psychomotor and developmental delay, hypotonia, seizures, microcephaly, characteristic facies, congenital heart diseases, bone defects and genital anomalies.

More than 70 cases were reported in the literature showing variable expressivity.

We report a 3 years old male patient, the only child of an apparently normal, young, unrelated couple. He was born naturally, at term, after an uneventful pregnancy (Wt 2500g, Ht 47 cm). Postnatal development was delayed. One episode of seizures was recorded (1 year old). Physical examination performed at 3 years 4month revealed: growth retardation (Ht - 2 SD, Wt - 1,76 SD), microbrachycephaly, dysmorphic face (round face, upslanting palpebral fissures, hypertelorism, epicanthic folds, broad nasal bridge, long philtrum, downturned mouth, thin upper lip, cupid's bow, low set ears with preauricular pits), short neck, clinodactyly of IIInd and IVth fingers, hypospadias, cryptorchidism (operated), sacro-coccygeal sinus, hypotonia, developmental delay. Echocardiography identified an ASD. Abdominal echography was normal. Based on the association of multiple birth defects, a karyotype was indicated. Chromosome analysis revealed a terminal deletion (1)(q42.1qter); parental karyotypes were normal.

In conclusion, we present this case to illustrate this relatively rare chromosomal abnormality and to compare the data of our patient with those presented in the literature

P0197. A large gap between age at diagnosis by screening and by symptoms in parent/offspring pairs with autosomal-dominant dilated cardiomyopathy

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Idiopathic dilated cardiomyopathy (IDCM) is inherited in about one third of cases. The new strategy of diagnosis familial diseases by means of the serial clinical screening of first- and second-degree relatives has provided evidence that the disease may be diagnosed in as many as 6-10% of the asymptomatic relatives. We document a large gap in age between symptom-based and screening-based diagnoses of AD-DCM in 26 pairs/triplets of parents and offspring from 23 families. IDCM was diagnosed according to WHO criteria. The inclusion criteria were: 1)two contemporarily affected relatives from a same family: one parent and one of the offspring; 2)first diagnosis and monitoring of all pairs/triplets of relatives at our Centre; 3)one of the pair/triplet of patients diagnosed by screening. The proband was the parent in 16 families with 17 pairs and two triplets of affected parent-offspring (group A), one of the offspring in seven families with five pairs and two triplets of affected members (group B). In group A, the clinical and functional findings were significantly more severe in the probands than in the relatives diagnosed by screening, but this was not the case in group B where the clinical worsening of the disease was rapid. In pairs/triplets parent-offspring contemporarily diagnosed as having AD-DCM, there is a one-generation gap between a symptom-based and screening-based diagnosis. These data suggest that DCM has a long-lasting asymptomatic phase in pairs in which the proband is the parent, may be genetically anticipated in pairs in which the proband is one of the offspring.

P0198. Dysgerminoma (seminoma), gonadoblastoma and mixed germ tumor in two adult 46,XY females after estrogen-progesterone treatment initiated at their 16 years of age

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We report two XY females, 19- and 25-year-old, with clinical signs of pure gonadal dysgenesis. Both patients underwent a laparoscopic gonadectomy. Histological examination of the gonads showed in each case coexistence of gonadoblastoma and dysgerminoma. Additionally in gonads of the first patient mixed germ tumor elements were found. PCR analysis showed no deletion of Y chromosome specific sequences in lymphocytes and fibroblasts of gonads in both cases. In the first case nonsense point mutation in the codon 81 within HMG-domain of the SRY gene [SRY, GLU81TER] was diagnosed. The mutation was excluded in the father of this patient. In the second case the SRY gene was apparently normal in lymphocytes of peripheral blood as well as in

gonadal fibroblasts. SRY gene mutations are observed only in no more than 15% of patients with XY-gonadal dysgenesis and any of them is clearly connected with the risk of gonadoblastoma. The hypothesis of GBY locus existence has no direct evidence in cases of dysgenetic gonad tumors.

We discuss that persistence of germ cells as well as sex cord elements in some cases of dysgenetic gonads. It may have the character of linear probability because of SRY presence. In consecutive cases the cellular components may undergo tumorigenesis under the influence of genetic as well as hormonal factors.

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P0199. Gingival Fibromatosis, sparse hair and congenital glaucoma: broadening the phenotype?

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Isolated gingival fibromatosis is generally a sporadic condition, but occasionally is seen in families. In this case, the onset is within the first ten years. Today, many patients had been described in literature, either presenting with gingival fibromatosis as an isolated feature or in association with other signs.

Jorgenson (1971) reported an adult female with gingival fibromatosis who also had sparse hair, nystagmus, and myopia.

Here we present a girl who suffers from gingival fibromatosis, which is also affected by unilateral glaucoma and localized sparse hair. Karyotype on blood cells and gingival fibroblast is 46,XX.

We propose our patient represents an association of feature helpful in refining phenotype of this condition.

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P0200. Cytogenetic investigation of POF subjects in Coimbatore District, Coimbatore, India.

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Human infertility has become a growing problem in the world today. Infertility is a major public health problem in India and it has also been estimated that in India between 10-15 % of the couples are infertile.

Premature Ovarian Failure (POF), is a common form of ovulatory dysfunction. POF, premature menopause, or early menopause, is a condition characterized by amenorrhea, hypoestrogenism, and elevated serum gonadotropin levels in women younger than 40 years. Premature failure (POF) is a "triad of amenorrhea, hyperandrogenism and hyperestrogenism and loss of fertility in women under the age of 40 years". POF is a typical feature in Turners syndrome. The most common etiology of gonadal dysgenesis is X monosomy (45, X), occurring in approximately 50% of individuals with the premature ovarian failure. A total number of 250 women were screened for infertility and nearly 144 patients showing Ovulatory Disorders were selected. Of them, 62 of them were with Premature ovarian failure. The 62 subjects presented with POF were presented with different types of chromosomal aberrations as, 45,X0 ; 47,XXX ; 45,X0/46,XY ; 46,XX,t(1p- ; 11q-); 46,XX,del(Xq); 45,X0,22s+; 45,X0/46,Xi(Xq) ; 46,XX,t(8p- ; 9q+); 46,XX, t(6p+ ; 15q-). An major event of this investigation is to assess X chromosomal alterations in infertility related to women population of this part of the country and to determine if chromosomal abnormality corresponds with Ovulatory disorders and to assess whether either predicts clinical outcome in ovarian disorders.

P0201. Frequency of Y chromosome microdeletions in Iranian males with idiopathic infertility

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Among couples with infertility problems, the male factor is the etiology in almost 50% of the cases and nearly 20% of all infertile men have idiopathic oligozoospermia. Recent studies reported that DNA

microdeletions on the long arm of the Y chromosome (Yq) may be responsible for some cases of idiopathic male infertility. The most important region of Yq is within intervals 5 and 6, which is designated the azoospermic factor (AZF). There are four subregions of this factor, named AZFa, AZFb, AZFc and AZFd, which we examined in our study to determine the frequency of Y chromosome microdeletions in Iranian males with idiopathic infertility. Microdeletions at the AZFa, AZFb, AZFc/DAZ, and AZFd regions were examined in 454 males with oligozoos-/azoospermia using PCR. Our results showed that 32 out of 454 subjects (7%) had Yq microdeletions: 30 with the AZFc/DAZ, 5 with AZFa, 5 with AZFb and 4 with AZFd microdeletion were found. Three patients with AZFabcd, two with AZFab, and one with AZFcde microdeletions were detected. Since microdeletion on the Y chromosome, especially at its AZFc/DAZ region, may be a major cause of oligozoos-/azoospermia resulting in male infertility in Iran, it is recommended that infertile men have Y chromosome microdeletion detection before *in vitro* fertilization.

P0202. Premature ovarian failure (POF) in a mother and a daughter with a balanced translocation t(X;11)(q24;q13)

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Premature ovarian failure (POF) is defined as the complete cessation of the menstrual discharge before the age of 40. The condition affects 1% of women and is heterogeneous. Several X;autosome translocations have previously been associated with POF. Here we report a mother and a daughter with a balanced translocation t(X;11)(q24;q13) and POF.

Physical mapping using FISH localised the Xq24 breakpoint to within a gene not previously reported as associated with POF. Similarly, the 11q13 breakpoint was mapped to a 70 kb region containing a gene which is expressed in the ovaries. We hypothesize that a subnormal transcription of both genes may contribute to POF in the two patients. Further studies are in progress to characterise the two breakpoints, the X-inactivation pattern and to clarify the role of the affected genes in POF.

P0203. arthritis in two children with chromosomal abnormalities

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Association of chromosomal abnormalities with arthropathies has been described before. In the present study, two children with juvenile rheumatoid arthritis and a chromosomal abnormality are presented. Patient A is a 9 years old girl with 18q minus syndrome and IgA deficiency. At birth she had dysmorphic features (hypertelorism, upslanting fissures, small mouth and chin, dysmorphic ears) and secundum atrial defect. Karyotype and telomere investigation revealed 18q minus syndrome. At the age of 5 years, she developed painful swelling of her left knee. Within next months arthritis of her both ankles, wrists, hands and feet was added to her clinical picture. One year later she developed joint contractures. Patient B is a 9 years old child with developmental delay, autism, dysmorphia (blepharoptosis, small nose, low set ears) and joint hypermobility. At the age of 7 years, she developed fever, arthritis of her knees and of her both hands and toes of small joints. Karyotype was 47XX+15(pter-q13) and investigation of PWSR was normal.

Conclusions: Juvenile arthritis in 18q minus syndrome has been described in 4 patients, but has not been connected with chromosome 15, previously. Further investigation may expand our knowledge as far as the probable existing association between chromosomal abnormalities and juvenile arthritis.

P0204. The frequency of chromosome 22q11.2 microdeletion in a group of 48 patients with conotruncal heart anomaly depending on the qualification criteria for FISH analysis used

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DiGeorge/velo-cardio-facial syndrome (DG/VCF, CATCH 22) is the most frequent observed chromosomal microdeletion syndrome. It is a contiguous gene syndrome usually connected with conotruncal heart anomalies, characteristic facial dysmorphic features, velopharyngeal insufficiency, hypoplasia/aplasia of the thymus with T-cell deficiency, hypoplasia/aplasia of parathyroid glands with hypocalcaemia and 1,5-3 Mb deletion on chromosome 22q11.2.

Congenital heart diseases occur in approximately 8 out of 1000 live births and conotruncal malformations account for about 50-60% of congenital heart defects diagnosed during the neonatal period. Recently many authors have proved that chromosome 22q11.2 deletion is very uncommon in isolated conotruncal heart defects. The percentage of cytogenetically confirmed cases increased dramatically if the heart defect was accompanied by at least one additional manifestation of the DG/VCF phenotype. In our study we examined 48 children with conotruncal defects and accompanying extracardiac anomalies or dysmorphic features. The aim of the study was to establish qualification criteria for the fluorescent 'in situ' hybridisation (FISH), in order to assess the diagnosis efficiency depending on the qualification criteria used. FISH was performed using DiGeorge DGCR2("N25", CLTD) region probe. We observed the best diagnostic rate in case of patients with conotruncal heart anomaly, velopharyngeal insufficiency and characteristic facial dysmorphism (22q11.2 microdeletion was confirmed in 74% of cases). However, taking into account the possibility of complications connected with immunity deficiency and hypocalcaemia we suggest that testing for the 22q11.2 microdeletion in newborn and very young children with conotruncal anomalies and a mild dysmorphic features or extracardiac abnormalities should be always recommended.

P0205. Hemiduplication - A Dysorganization-like syndrome?

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The mouse mutant disorganization phenotype was first described by Hummel [1958, 1959]. It arises spontaneously and disrupts the orderly processes of development. It causes bizarre and variable developmental anomalies, such as mirror-limb duplications; hamartomatous skin papillae, and other internal organ abnormalities.

We report the autopsy finding in a fetus with hemiduplication of most body organs.

A 29-year-old primigravida woman presented with ultrasonographic findings of 2-vessel cord, bilateral club feet, and right diaphragmatic hernia.

The couple is non-consanguineous. The pregnancy was uncomplicated. The fetal karyotype was 46,XX. The autopsy showed right hemifacial microsomia and hemiatrophy of the right trunk, labia majora, sternum and ribs. There was duplication of the right arm, to the mid-humerus level, right clavicle, right scapula, part of the right iliac wing, right adrenal gland, right kidney, right lung, stomach and most of the small bowel. The duplicated right kidney revealed multicystic dysplastic changes. There was a cloacal tag and malposition of the anus, right diaphragmatic hernia, pulmonary hypoplasia and deviation of the heart to the left. The uterus was bicornuate, the pancreas was annular and there was a segmentation defect of the upper thoracic spine. There were 11 pairs of ribs, bilateral talipes equinovarus and left sandal gap. The right leg was shortened with a right knee and right elbow pterygia and a single umbilical artery. The spinal cord showed myelodysplasia

and the gray matter was disorganized.

The above findings are unique and represent a variant of the mouse DS or a whole body twining.

P0206. 6q terminal deletions: presentation of two new cases.

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Deletions of the distal part of the long arm of chromosome 6 have not been associated to a well distinctive and recognizable phenotype, even if an emerging '6q- terminal deletion syndrome' starts to be delineated.

In order to verify this, we compared the phenotype of two new cases with the cases described in literature. A common phenotype emerged from these reports, including psychomotor retardation, growth retardation, hypotonia, seizures, and 'typical' facial dysmorphisms, along with various non specific malformations. These differences can be ascribed to a different deletion extent or in difficulties in defining precisely the breakpoints on the basis of only classic cytogenetic studies.

In our patients the deletion extent was further analyzed by molecular-cytogenetic techniques: in both of them the breakpoint was located in an interval of about 442 Kb, between the clone RP11-150P20 (present) and the clone RP11-152P19 (deleted). The breakpoints fall within the fragile site FRA6E, the 'centre' of which is overlapping with the PARK2 gene, that spans the telomeric half of the fragile site. A potential role of fragile sites in generating terminal deletion is suggested.

The 8 Mb deleted region is known to contain several genes; two of them, PARK2 and TBP, are known to be associated with the autosomal recessive form of juvenile parkinsonism; and the autosomal dominant spinocerebellar ataxia type 17 (SCA 17), respectively. All the cases reported were pediatric, thus it is not known if any of them has shown these neurological manifestations during years.

P0207. A new locus for brachydactyly type A2 maps to chromosome 20p

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Brachydactyly type A2, an autosomal dominant hand malformation, is characterized by short and laterally deviated second and fifth fingers. Also, the first and second toes can be affected in a similar way. Heterozygous missense mutations in the gene coding for bone morphogenetic protein receptor 1b (BMPR1B) were shown to cause brachydactyly type A2 in some cases by acting in a dominant negative manner. Recently, we performed a clinical study in a large Brazilian pedigree presenting with brachydactyly type A2, including radiographic evaluation of hands, feet and spine. We further undertook a linkage analysis using the SNP-GeneChip Human Mapping 10K Array/Assay Kit from Affymetrix. Additional polymorphic microsatellite markers were used for the fine mapping to narrow the defined region. Using this approach, we mapped a new locus for brachydactyly A2 to a 17 cM region on chromosome 20p. As a candidate gene located within this new locus, we first sequenced the coding region of BMP2, known as an important ligand of the BMPRs that mediates essential functions in chondrocyte differentiation and bone formation, but no mutation was found. Several other candidate genes located within the disease locus will now be sequenced.

P0208. Apparent intergenerational stability of CTG trinucleotide repeats in three generations of women with myotonic dystrophy.

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Myotonic dystrophy (MD) is a frequent neuromuscular affection due to an unstable CTG trinucleotide repeat (CTGtr) located in the 3' untranslated region of the dystrophia myotonica protein kinase (DMPK) at 19q13.3.

Normal subjects have 5 to 37 CTGtr copies which remain stable following intergenerational transmission while affected patients have over 50 CTGtr. In a majority of DM patients, the number of repeats is highly unstable and increases during parent-offspring transmission of the mutant allele, providing molecular basis to the anticipation phenomenon (increased severity of the disease in successive generations).

We report on apparent CTGtr stability in three generations of women with MD. The number of the CTGtr in blood cells was estimated at 133, 121 and 109 for the grandmother, mother and daughter, respectively. We had the opportunity to analyse the CTGtr in several fetal tissues after termination of the pregnancy of the 121 CTGtr carrier woman for an affected fetus at 15 WG. The size of the CTGtr (n = 113) was strictly identical in muscle, brain, liver and heart and chorionic villi as well. These data suggest the existence of a dominantly inherited factor preventing the instability of the expanded CTG stretch during female gametogenesis in this family. Studies of some candidate genes are in progress.

P0209. A BAC tiling array for human chromosome 21: comparative genome hybridization (CGH) to map partial trisomies, deletions and translocations, and insertion-deletion polymorphisms

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A major goal of Down syndrome (DS) research is to identify which genes or other functional genomic elements are responsible for particular aspects of this complex syndrome. Central to this is the concept of critical regions (CRs). That is, regions of Hsa21 which, when present in 3 copies (aneuploid), result in one or more of the characteristic features of DS. Based on rare individuals with partial aneuploidy, CRs have been mapped for several features of DS. However, the extent of these regions have not been determined in detail. Studies of other rearrangements (for example, deletions and translocations) involving Hsa21 may also provide information on the contribution of Hsa21 genes to DS.

In order to provide high resolution mapping of pathogenic partial aneuploidies, deletions and translocations, and to identify polymorphic insertion-deletions, involving Hsa21, we have made a BAC array covering 21q. The array consists of 411 Hsa21 BACs (from RPCI and CTD libraries), with a mean overlap of 85kb giving an approximately 2-fold tiling path. The array also includes approximately 100 BACs for normalization and controls. This CGH array will be used to assess the Hsa21 content of partial trisomy cases of DS to aid in the understanding of genotype-phenotype correlations. In addition, we will characterize the extent of Hsa21 large-scale copy number variation which was recently identified in the human genome. More than 50 samples with Hsa21 genomic abnormalities are available to us and their analysis is in progress.

P0210. Cohen syndrome: molecular analysis of the COH1 gene in the Italian cohort of patients.

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Cohen syndrome is a rare autosomal recessive condition characterized

by mild to severe mental retardation associated with microcephaly and truncal obesity. Characteristic dysmorphic features are down slanting palpebral fissures, short philtrum, open mouth, prominent upper central incisors, prominent nose. Beside the facial gestalt, major diagnostic criteria include retinal dystrophy and neutropenia. Cohen syndrome is a quite rare condition overrepresented in the Finnish population, with a highly homogeneous phenotype. In other populations clinical features may be remarkably different. We have collected a cohort of 15 Italian patients, originating from different regions. A diagnostic rating of 'certain' (5 patients), 'probable' (4) and 'possible' (6) was assigned on the basis of clinical criteria. DHPLC mutation analysis of the *COH1* gene, recently identified as responsible for Cohen syndrome is ongoing. Analysis of the first 8 exons revealed three truncating mutations in heterozygous state. Two are novel mutations: p.Q721fsX744 and p.R1143X. One, p.R2535X, is a recurrent mutation previously identified in the German population. The mutation, p.C1117fsX1124, prevalent in the Finnish population, was not found. The above mutations were found in three patients who are likely genetic compounds as expected by the absence of consanguinity in the Italian cohort. Two patients were classified as 'certain' and one as 'probable' on the basis of clinical criteria. Presently, no mutations were found in the group of 'possible' Cohen although only 8 out of 62 exons were analyzed. Extension of the analysis to the entire coding sequence will allow to define Cohen syndrome clinical features in the Italian population.

P0211. Unusual observations associated with novel C-terminal *MECP2* mutations

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We report novel C-terminal *MECP2* mutations. One was a frameshift deletion, 1135_1142delCCCGTGC, inducing a premature stop codon in a 19-year-old woman with mental retardation and epilepsy. Preservation of language capabilities, purposeful hand use and sufficient locomotion implied an atypical variant of Rett syndrome. Occipito-frontal head circumference was large at birth (36 cm; SDS = 1.7) and increased to macrocephaly in the adult patient (58.5 cm; SDS = 2.3). Thus, head size and head growth appear to be of limited reliability in the diagnosis of *MECP2*-associated phenotypes.

In a 3.7-year-old girl who presented with classical signs of Rett syndrome such as delayed head growth, stop of language development, and ataxia, four C-terminal *MECP2*-mutations were detected: an in-frame deletion of 3 nucleotides, 992_994del3bp, resulting in the removal of the lysine at position 331, a one-nucleotide frameshift deletion, 1027delG, a missense mutation 1061G>C, and a second frameshift deletion, 1160_1193del34bp. None of these mutations is contained in the *MECP2* mutation databases. The 1061G>C mutation that causes an arginine to leucine substitution was present in the non-affected father of the patient indicating that this mutation probably is a normal variant.

Our observations indicate a large variability associated with C-terminal *MECP2* mutations.

P0212. Is there a novel locus for Hirschsprung disease(HSCR) on chromosome 15q11q14?

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Hirschsprung disease (HSCR, MIM # 142623) is a major cause of functional intestinal obstruction with an incidence of 1:5.000 live births. HSCR is considered a multifactorial malformation with low and sex dependent penetrance and variable expression depending on the length of the aganglionic segment. At least 8 major genes have been identified to be involved in HSCR. Interstitial chromosomal deletions in combination with HSCR lead to the identification of such genes like *RET* (10q11.2), *SIP1* (2q22q23) and *EDNRB* (13q22.1q32.1). According to our knowledge, chromosome 15q11q14 has not been associated so far with HSCR.

We report about a now 16 months-old girl with HSCR and major features of Prader Willi syndrome (PWS, MIM# 176270) associated with a de novo paternal deletion 46,XX,del(15)(q11.1q13/q14)(D15Z1x2,SNRPNx1,UBE3Ax1,PMLx2). Genotyping identified hemizygosity of 2 microsatellite markers (D15S1048; D15S976) with loss of the

paternal allele. Heterozygosity and biparental inheritance was proven for D15S1007 at 15q14 and all other distal loci. Thus, approx. 15-20 Mb of proximal 15q including PWS region is deleted. So far no gene for HSCR has been identified within chromosome 15q11q14. HSCR in our patient might be related to hemizygosity of the deleted segment. However, none of the rare patients with comparable deletions (Windpassinger et al, 2003) demonstrate HSCR. Alternatively, one of the breakpoints may have interrupted sequences important to prevent formation of HSCR. Significant might be the recent mapping of a novel locus for primary ciliary dykinesia (CILD4, Jeganathan et al., 2004) to a 17cm critical segment overlapping with our distal breakpoint region.

P0213. Skewed X-inactivation in a family with mental retardation and *PQBP1* mutation

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We previously reported on a family with mentally retarded males, obligate carriers with skewed X-inactivation and the gene responsible for this condition mapping in the Xp11-q12 region. Here we present more clinical and molecular data within an expanded pedigree segregating a known four-bases deletion in the exon 4 of *PQBP1* gene. The unusual X-inactivation pattern of the obligate carriers in this family raises the topic about a possible modulation of the carrier's phenotype by the X-inactivation status. Mental impairment within the affected males is widely variable, ranging from borderline intelligence to severe mental retardation. These data support the search for *PQBP1* mutations when mentally retarded males in a family kindred compatible with X-linked inheritance show microcephaly, short stature, spastic diplegia/brisk reflexes.

P0214. Screening of mutations in the human *NIPBL* gene in patients with Cornelia de Lange syndrome by DGGE.

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Cornelia de Lange syndrome (CdLS) is a clinically heterogeneous autosomal-dominant developmental disorder characterized by upper-extremity malformations, cardiac defects, growth retardation and gastrointestinal abnormalities. Additionally, typical facial features include rotated low-set ears, long eyelashes, thinner upper lip and depressed nasal bridge are found in patients.

The prevalence of CdLS is estimated to be around 1/10,000. Most cases are sporadic, although several family cases are described. Recently, it was documented that CdLS is caused by missense and nonsense mutations in the human *NIPBL* gene. *NIPBL* is the human homolog of the *Drosophila* Nipped-B gene which is involved in Notch signaling.

The human *NIPBL* gene consists of 47 exons. Mutations were found in the entire coding region (Gillis et al., 2004).

To identify mutations in German CdLS patients we established a DGGE-screening protocol for detection of mutations in all exons of the *NIPBL* gene.

So far, we screened 20 German patients with suspected CdLS. Our preliminary data indicate that DGGE is a highly sensitive screening method for detection of mutations in the *NIPBL* gene.

P0215. Hemispheric polymicrogyria in a patient with a Sturge-Weber-like Syndrome

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Sturge-Weber syndrome is a rare neurocutaneous disorder characterized by facial cutaneous vascular malformations and leptomeningeal angiomas. Associated features include seizures or other neurologic consequences and glaucoma. We report here the case of a 3½-year-old girl born to consanguineous Amish parents. She presented with midline and right forehead port-wine

stains, left-sided weakness demonstrated by right hand preference, first noted at 6 months, as well as eversion and rotation of the left foot. She developed seizures, responsive to carbamazepine, at the age of 2½ years. Physical examination showed no other dysmorphic features and appropriate growth. Imaging of the brain showed right hemispheric dysgenesis most prominent in the frontal lobe with an irregular microgyric pattern and apparent cortical thickening in the frontal, parietal and anterior temporal lobes, typical of polymicrogyria. There was reduced white matter underlying the polymicrogyria. The association of polymicrogyria in association with Sturge Weber syndrome has been rarely reported and may provide insight into the pathogenesis of polymicrogyria, suggesting an association with abnormal cortical vasculature during development.

P0216. Is there a specific phenotype associated with 3p14 microdeletion?

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We report here a 26 year-old male patient with mental retardation and dysmorphic features. Facies is characterized by high forehead, narrow horizontal palpebral fissures, epicanthic folds attenuating with the age, broad nasal bridge, bulbous and broad nasal tip with prominent columella, slightly posteriorly angulated ears of normal length with thick helix and hypoplastic lobules. He has a normal head circumference. He also has very small hands and feet and pes planus, contractures of finger joints and clinodactyly of the 5° finger. Language is absent. A brain MRI, performed at 14 years, showed hyperplasia of corpus callosum. During the examination he shows an aggressive and self-injuring behavior. Standard karyotype was normal. Whole genome array-CGH analysis revealed a deletion of about 8 Mb of the short arm of chromosome 3. High resolution karyotype confirmed the presence of an interstitial deletion: 46,XY,del(3)(p14.3;p14.1). Interstitial deletions of proximal 3p were reported in other 13 cases. All of them were identified by standard karyotype. Two are slightly larger deletions totally missing the band p14 and the other partially overlap extending toward the centromere. This is the smaller reported deletion. Some dysmorphic features like high forehead, epicanthic folds and narrow horizontal palpebral fissures seem to be consistent with deletion of this region. The finding of these features in a patient should represent a clinical hint for deeper investigation of proximal short arm of chromosome 3. Widely use of array-CGH may also lead to the identification of additional microdeletions, further delineating a specific syndrome.

P0217. Mutational analysis of NSD1 gene in Finnish patients with Sotos syndrome

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Sotos syndrome (OMIM #117550) is a rare childhood overgrowth syndrome characterized by pre- and postnatal overgrowth, typical facial appearance, advanced bone age, developmental delay and predisposition to cancer. Although most cases are sporadic, few families with autosomal dominant inheritance have been reported. Heterozygous point mutations in the NSD1 gene or a microdeletion covering the entire gene are the major cause of the syndrome. There is no hot spot for mutations and to date there are over hundred different published pathogenic mutations in the NSD1 gene.

Here we report the results of a mutation analysis using denaturing high-performance liquid chromatography (DHPLC) and/or direct sequencing of the NSD1 gene in 17 patients with clinically diagnosed Sotos syndrome. All cases were sporadic but only in two cases we had parental samples. Out of the 17 patients 15 (88%) were found to carry a unique heterozygous mutation. Eleven of these mutations resulted in a truncated protein product and four resulted in amino acid changes. Twelve mutations are novel and three have been published previously. In the analysed families mutations had occurred *de novo*. Our results are concordant with the results of the previous studies, which have shown that in European populations point mutations are the major cause of the

syndrome. Currently we are developing a quantitative real - time PCR method to detect also small deletions, to further clarify the mutational spectrum in Finnish patients, and improve the molecular genetic testing of the syndrome. Genotype - phenotype correlation will be discussed.

P0218. Cumming syndrome: report of two affected sibs

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In 1986 Cumming et al. described a stillborn male infant, born at 27 weeks' gestation who had bowed limbs, marked cervical lymphocele, polycystic dysplasia of the kidneys, pancreas and liver and polysplenia.

Subsequently Urioste M. et al 1991 and Perez del Rio et al. 1999 reported four cases with similar findings. We have recently described (Am J Med Genet 2005) a 46,XX fetus with ascites, campomelia, multicystic dysplastic kidneys, polysplenia, absence of fallopian tubes and uterus and laterality defects of the heart and lungs suggesting the diagnosis of Cumming.

The patient was the first-born of healthy, non consanguineous Caucasian parents. Family history was unremarkable. Ultrasound examination at 22nd week showed a thoracic hypoplasia, ascites, mild hyperchogenic kidneys, oligohydramnios, shortening and bowing of the lower limbs. The fetal stomach was not visualised and there was a single umbilical artery was noted. Fetal blood chromosomes were 46,XX. The pregnancy was terminated. During the following pregnancy ultrasound at 16th week showed oligohydramnios, hyperechogenic kidneys, bowing of the lower limbs. Cytogenetic analysis on AL showed normal male karyotype. Pregnancy was terminated. Pathology: on external examination the fetus showed a cystic hygroma, ascites. Bowing femura and bilateral talipes equinovarus were present. Kidneys showed diffuse multicystic dysplasia. The external genitalia were male and testes were in abdomen. Other organs were normal in number and structure.

The findings of our cases are similar to those reported by Cumming et al (1986). The recurrence of a similar clinical picture in two sibs supports the autosomal recessive inheritance of the syndrome.

P0219. Auditory function in Bardet-Biedl patients

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Introduction: Recently, the pathophysiology of Bardet-Biedl syndrome has been described as a primary ciliary dysfunction disorder. Ciliated cells are present in the inner ear.

Methods: A cohort of 21 patients with Bardet-Biedl syndrome genotyped for 8 BBS genes was studied. Patients were evaluated for the auditory function with audiograms and otoacoustic emission (recording of vibrations created by contraction of outer hair cells).

Results: Audiograms showed normal hearing. The threshold was inferior to 20dB for twenty patients but was higher than expected for the age. One patient had moderate hearing loss. Otoacoustic emission was altered for 55% of the patients (31% absent and diminished for 24%).

Discussion: The selective alteration at the level of the outer hair cells, revealed by the OAE may be due to a defect at the level of the kinocilium, a primary cilia (9+0) found at the top of outer hair cell bundle, probably necessary for the cellular organisation the bundle. No specific genotype -auditory phenotype correlation was identified to date.

P0220. Recurrence of Achondrogenesis type II caused by a dominant COL2A1 mutation and "patchy" expression in the mosaic father

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Achondrogenesis type 2 (ACG2) is produced by dominant mutations in COL2A1. These occur *de novo* in the vast majority of cases. In rare cases, mutations leading to Kniest dysplasia in children have been found at mosaic state in one of the parents who fitted the diagnostic criteria for Stickler syndrome or mild spondylo-epiphyseal dysplasia (Winterpacht et al, 1993; Spranger et al, 1994). In another family, recurrence of ACG2 in two fetuses of an healthy couple was interpreted as evidence for gonadal mosaicism (Faivre et al, 2004).

We present a family in which three fetuses were affected by ACG2. Both parents were of normal height, but the father had had scoliosis as a child and showed slight body disproportion with short trunk as an adult. His femoral heads, pelvis and lumbar vertebrae (telltale sites for collagen 2 disorders) were normal on xrays; only some thoracic vertebrae showed mild flattening and anterior wedging. Although a somatic mosaicism was considered unlikely, molecular analysis revealed a COL2A1 point mutation (G1037T) in the fetuses, and a signal consistent with mosaicism in father's blood DNA.

This evidence reinforces the need to consider mosaicism in families with newly recognized COL2A1 disorders and indicates that somatic mosaicism can lead not only to milder but generalized clinical phenotype but also to regional ("patchy") expression, with some bones affected and others entirely unaffected. This may depend by the time of onset and the body region or segment in which the somatic mutation arises.

P0221. Results of prenatal diagnosis of thalassemia in the Iranian province of Hormozgan (2001-2004)

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We review 126 persons (63 couples) with hemoglobinopathy attending two genetic counseling centers in Hormozgan province, which is located in the south of Iran in the north of the Persian Gulf, for the years 2001- 2004. After genetic counseling these couples were referred for molecular investigations. Results are as follows: 122 persons (96.8%) were Fars, four (3.2%) were Baloochis. Thirty eight couples had consanguineous marriages (61.3%). Twenty couples that were referred to us had an affected child. Among the patients who were referred to us, 111 individuals had beta thalassemia minor while 15 other individuals were carriers for other hemoglobin disorders such as alpha thalassemia, hemoglobin D and hemoglobin S. The mutation spectrum reveals IVS1-5 (76 cases) as the major beta thalassemia mutation in our province followed by IVSII-1 (11 cases), IVS1-110, IVS1-6, C5, C15, C30. Two types of sampling were performed for prenatal diagnosis: CVS (chorionic villus sampling) and amniocentesis. In 50 cases prenatal diagnosis was done. The results were as follow: minor thalassemia: 26 fetuses (52%); normal homozygote: 11 fetuses (22%), major thalassemia: 13 fetuses (26%). Our result shows a Mendelian distribution in our prenatal diagnostic program in Hormozgan province.

P0222. Organising the management of patients with Fabry disease in the view of multidisciplinary approach

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Objective: Fabry disease is a rare lysosomal storage disorder. The management of Fabry disease is very complex, included the clinical and molecular genetic diagnosis, the assessment of disease severity, the genetic counselling and the enzyme replacement therapy (ERT).

Methods: The complexity of the management has given the reason for established Fabry Work Group in our Institute. Through a case

report we discuss the relevant clinical features, the molecular studies as well as our experiences with the ERT. Different levels of medical attendance will be also presented.

Case report: A 27 years old male patient was admitted to our department with angiokeratomas and recurrent diarrhoea. The diagnosis of Fabry disease was confirmed with the decreased plasma activity of GAL. The corneal involvement was revealed by slit-lamp examination. Cardiac manifestation was ruled out. Normal renal function was observed. We performed reverse transcription-polymerase chain reaction analysis using RNA isolated from whole-blood sample. The amplicons were subcloned and sequenced. A novel missense mutation was identified in the 266. codon (5. exon) of GAL: GAT (Asp) → TAT (Tyr).

After 12 months of ERT the patients reported improvements in the ability to sense heat as well as in his general well being. Decreased numbers of diarrhoea were occurred. New organ involvement or progression was not observed.

Conclusion: ERT should be started in the early stage of the disease, before the irreversible complications are developed. To get this optimal point, cooperation of paediatricians, cardiologists, nephrologists, neurologists, ophthalmologists, dermatologists and molecular biologists should be performed.

P0223. Deafness : clinical and genetic evaluation of 102 patients.

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Deafness is the most prevalent (1/700) sensory defects in humans. It is an etiologically heterogeneous trait with many known genetic and environmental causes.

We reviewed medical records from 102 patients who have been seen in our department of clinical genetics over 6 years. We analyzed clinical features of deafness : isolated or syndromic form, type and severity of hearing impairment, age of onset, uni or bi-lateral impairment, sporadic or familial case, and looked to medical investigations they had : presence of any malformation and/or dysmorphic features, X-rays of inner ear, renal, cardiac, thyroïd, visual, physical and mental development, chromosomal and/or genetic screening.

Out of 102 patients, there were 40 adults and 62 children. Sex ratio was 1.37 F/M. Half of them (49 %) had a syndromic form and 53 % had an isolated deafness. Out of the patients with syndromic forms, we could identify 15 Usher, 4 type I Waardenburg, 3 Goldenhar, 4 B.O.R. syndromes. One had a partial chromosomal del 2q. Out of 53 nonsyndromic cases, 16 were sporadic and 37 were familial. Transmission was clearly autosomal dominant (DFNA) in 13 cases and autosomal recessive (DFNB) in 10 cases. Seven cases were homozygous for mutations in GJB2 and 5 composit heterozygous for a mutation in GJB2 and a mutation in GJB6. One familial case with a maternal transmission was due to the T7511C mitochondrial DNA mutation.

We found few studies with such an approach of a global population of deaf patients. We will comment ad compare our results.

P0224. The effects of pentoxyfilline on drug allergy induced by α -galactosidase

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Objective: Pentoxyfilline is widely used to treat peripheral vascular diseases. Additional to this well-known indication, pentoxyfilline is a promising new agent for the treatment of SIRS (severe immune response syndrome). Pentoxyfilline decreases release of TNF- α , the central mediator of SIRS. It also inhibits cytokine-induced polymorphonuclear cell activation and adherence, as well as polymorphonuclear cell degranulation and superoxide anion production.

Methods: We report a case of a young male patient with Fabry disease in whom hypersensitive reaction developed against enzyme replacement therapy (ERT).

Case report: A 25-year-old male patient was admitted to our Department with angiokeratomas, post-stroke and pain syndromes. After the diagnosis of Fabry disease was confirmed, ERT was started. Since that, two episodes of adverse events were observed with severe

shiver and hypertension. The relationship between these events and the infusion were probable in both occasion. Although the pre- and post infusion IgE titres and the post infusion serum tryptase level were normal; the result of lymphocyte transformation test showed mild hypersensitisation against the drug. Regarding the patient's progressive multi-organ involvement and the above mentioned results of laboratory studies, continuation of ERT with pre- and post infusion pentoxyfilline administration was decided. The patient received 14 courses according to this protocol without any problem.

Conclusion: The clinical use of ERT is limited mainly by the treatment cost, the production of neutralising antibodies and the hypersensitive reactions caused by the antigenic effects of the drug. The presented method may be useful in the treatment of Fabry patients with hypersensitive reaction.

P0225. Carrier diagnosis of DMD/BMD deletions by QPCR

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We sought to find a technique to establish a direct DMD/BMD carrier diagnosis in families in which it was not possible to perform this kind of diagnosis.

We applied the QPCR technique by Light Cycler using the Fast Start DNA master SYBR Green (Roche) to muscular and brain promotors and to 25 different exons scattered over the dystrophin gene.

We diagnosed 64 women from 30 families presenting a risk of being DMD/BMD carriers. The women presented risk because they belonged to families in which a deletion in dystrophin gene was detected in the affected boy. The results were compared with the indirect analysis by STRs.

The Light Cycler technique offers reliability and speed in the heterozygous carrier diagnosis. Furthermore, this technique allows us to avoid the hybridization with specific probes to each exon.

Owing to this method it is possible to diagnose DMD/BMD carrier women in families in which other methods could not be applied or did not yield accurate results (sporadic cases, no informativity by STRs, unavailability of parents of the women studied).

P0226. Molecular analysis of CTDP1 gene in consanguineous families presenting typical or partial CCFDN phenotype

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Congenital Cataract Facial Dysmorphism Neuropathy syndrome (CCFDN) is a complex autosomal recessive disorder involving multiple systems and organs. Initially reported from the Roma Gypsy group originating from Bulgaria, CCFDN is characterized by developmental impairments affecting eyes and face, peripheral and central nervous system, growth and intellect. Recently, the disease causing gene was identified, CTDP1, encoding an essential component of the eucaryotic transcription machinery. So far, a unique founder homozygous mutation, g.IVS6+389C>T, has been identified in CCFDN patients, resulting in an aberrant splicing and causing partial loss of expression. We report molecular analysis of three consanguineous Gypsy families with either typical or partial CCFDN. The homozygous mutation was identified in families G and B. Indeed, although typical CCFDN phenotype was observed in family G, in family B, a 5 years old girl mainly presented a demyelinating neuropathy with congenital cataract although developmental delay and facial dysmorphism were slight. In family L, initially diagnosed as non syndromic CMT4, the same mutation was found to be heterozygous in one patient. Investigation of other loci already known to segregate in Gypsies (NDRG1, HMSN-R) and transcriptional studies from cultured lymphocytes from CCFDN individuals of these families are in progress. However, despite the homozygous presence of the mutation in one affected patient (family B), the clinical phenotype is somewhat different, suggesting that typical CCFDN might be misdiagnosed at lower ages. We thus propose CTDP1 as a candidate gene in patients presenting a combination of peripheral neuropathy and congenital cataract, even in absence of others developmental features.

P0227. The difficult task of sensorineural hearing loss (SNHL) in patients with idiopathic cardiomyopathies.

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SNHL may occur in patients with inherited cardiomyopathies both hypertrophic (HCM) and dilated (IDCM). To date, hearing loss has been reported in patients with mitochondrial DNA (mtDNA) defects related to HCM, or occasionally described in patients with autosomal dominant cardiomyopathies. Recently the epicardin gene (locus 6q23-24) has been linked to familial IDCM associated with SNHL. No mutations has been identified to date in the epicardin gene.

Aim of the study was analyzing mtDNA, epicardin, connexin26 (Cx26) and connexin30 (Cx30) in patients with cardioauditory syndromes, both HCM and IDCM associated with SNHL.

The mtDNA, Cx26, Cx30 and epicardin genes were analysed in 15 patients with SNHL, 4 with HCM and 11 with IDCM. MtDNA, Cx26 and epicardin genes were analysed by direct sequencing (ABI 3100 Genetic Analyzer); Cx30 deletion was evaluated by amplicon size.

We identified the A3243G and the G1644A heteroplasmic mutations in 2 of 4 HCM patients, both with familial matrilineal hearing loss and associated encephalomyopathy. The A3243G carrier also carried the heterozygous V37I mutation in the Cx26 gene. Of the 11 patients with IDCM, one was found to carry the heterozygous G22V mutation in the epicardin gene and one the Cx26 deletion (del30G). Of the remaining 9 patients with IDCM, none was carrier of mutations. A second mutation (D21E) in the epicardin gene was identified in one of 30 patients screened for SNHL. Genotyping process of cardioauditory syndromes should be based on clinical evaluation and addressed to the potential causative genes by the clinical ground.

P0228. Two novel cases of Taybi-Linder syndrome (MOPD I/III) in brothers: further delineation of the phenotype focusing on the cerebral malformations

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Microcephalic and osteodysplastic primordial dwarfism (MOPD) type I/III or Taybi-Linder syndrome is a very rare autosomal recessive syndrome (20 cases reported to date) of unknown cause, characterized by severe intrauterine growth retardation, dwarfism with short limbs and dislocated hips and elbows, dry skin, sparse hair and eyebrows and microcephaly. Malformations of the central nervous system were previously reported in Taybi-Linder syndrome but very few accurate and illustrated descriptions are available to date. Here, we provide a complete phenotypic description of two affected brothers born to first cousin healthy parents. Moreover, we show that, in both patients, supratentorial anomalies which include frontal polymicrogyria, agenesis of the corpus callosum and a large interhemispheric cyst are far more severe than the cerebellar ones that appear to be restricted to a mild vermis hypoplasia.

P0229. Anthropometric characteristics of the Polish group of patients with Nijmegen breakage syndrome (NBS)

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive disease characterized by microcephaly, growth retardation, immunodeficiency, radiosensitivity, and an elevated risk of cancer. NBS seems to be prevalent among Central and Eastern European populations, with Polish patients constituting approximately half of all registered NBS patients worldwide. All 84 Polish patients identified to date (Polish NBS Registry, December 2004) share the common founder mutation in the *NBS1* gene, 657del5. A total of 43 patients aged from birth to 20 years, observed at a single centre (Children's Memorial Health Institute), were enrolled in this study. A detailed

physical examination encompassing about 55 phenotypic traits was performed. Anthropometric measurements, repeated at 6-12 month intervals, included in addition to height and weight, 18 somatic traits of the trunk and limbs, and 15 traits of the head. On the basis of anthropometric parameters it was shown that the somatic development of the patients was not only significantly ($p<0.001$) delayed from the very earliest stages of life in comparison with age- and sex-matched controls, but was also characterized by a differentiated dynamic of ontogenesis. It was possible to identify three developmental stages in relation to height, weight, and chest circumference: delay (years 1-2 of life), acceleration (from age 2 to 10 years, depending on the parameter), and stabilization, and two in relation to head circumference (no acceleration).

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P0230. Split hand / foot malformation (SHFM) in two members of a family with monosomy 17p13.3 and 22q11.

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We report on a 30 year old man presenting with severe mental retardation, inverted nipples, glandular hypospadias and limb malformation consisting in monodactyly of both hands and lobster claw deformity of feet. Standard karyotype revealed 45,XY,der(17)t(17;22)(p13.3;q11.21)-22. The unbalanced rearrangement was also present in the proband's sister, a mentally retarded woman with unilateral breast hypoplasia and unilateral split foot, and in their mother who had mild learning difficulties but no limb malformations. Short stature, hypernasal speech and a characteristic facial dysmorphism with variable severity was present in all three.

FISH studies in the proband and his mother ruled out the deletion of *LIS1* in 17p13 and confirmed the deletion involving the DGS/VCFS region in 22q11.2 (*TUPLE* probe) in all cells. CGH study on a microarray with the tiling path of chromosome 22 in these two patients showed a deletion extending from the centromere to the most proximal 1.5 Mb within the critical DGS/VCFS region. To our knowledge, this is the first case of ectrodactyly (SHFM) in a patient with a chromosomal aberration involving either chromosomes 22 or 17. Only mild limb anomalies have been described in patients with the typical DGS/VCFS phenotype and deletion 22q11.2, and none of the 5 known SHFM loci map to either of these regions. Our data indicate that monosomy 22q10-q11.21 and/or 17q13.3-qter can lead to disruption of a pathway involved in the development of hands, feet, genitalia and breasts. Current studies aimed at identifying candidate genes for these anomalies are being performed.

P0231. Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2

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Clinical variability associated with the common 22q11.2 microdeletion is well known and led to broad application of FISH diagnostics with probes for loci *TUPLE1* or D22S75 (N25), although rarely reported atypical deletions associated with the same phenotypic spectrum would not be discovered by these probes. As most types of 22q11.2 deletions occurred between low copy repeats within the region (LCR22), we assumed that atypical deletions would be more common than reported. To address this question and the possibility of a deletion size related genotype-phenotype correlation, we used a set of 10 FISH DNA probes which is capable to detect all reported and hypothetical types of deletions in between the LCR22, and analysed a total of 350 patients.

Patients with conotruncal heart defects (ctCHD) or with typical VCFS phenotype showed the common 3 Mb and nested 1.5 Mb deletions

in 18.5 % and 78.6 %, respectively, but no atypical deletion, while 5 % of patients with mildly suggestive, atypical phenotype, showed atypical distal deletions, which were not detected in a control sample. These statistical significant differences demonstrate that atypical distal 22q11.2 deletions are very uncommon in patients with ctCHDs, while atypical congenital heart defects and mild dysmorphism is a recognizable feature of atypical distal deletions. Further phenotype-genotype analysis disclosed association of significant developmental delay with the distal part of the common deletion region and choanal atresia and atypical CHDs with the adjacent distal deletion region.

P0232. Mutational analysis in NF1 patients screened for heart abnormalities

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It is still unclear whether cardiovascular malformations are more common in the NF1 population. Studies so far performed have analysed data from multiple centres or from selected NF1 populations and prevalence figures have ranged from 2.3% to 27%. Heart involvement has been associated to more severe NF1 phenotypes and large NF1 gene deletions.

The aim of this study is to evaluate the spectrum of cardiovascular abnormalities in nine NF1 individuals screened for mutations in the NF1 gene. We studied the heart (by means of electrocardiogram, complete 2-dimensional echocardiograms and Doppler studies) and performed (by means of DHPLC and DNA sequencing) a mutational analysis of the whole NF1 gene in nine NF1 patients (4 males, 5 females; aged 3 to 28 years) seen at the Department of Paediatrics of the University of Catania and the National Research Council of Cosenza, Italy.

The results showed that none of the nine patients had or developed cardiovascular abnormalities. No large NF1 gene deletions have been detected, but the NF1 gene analysis revealed 1 stop mutation, 3 amino acid substitutions, 3 deletions, 1 insertion and 1 intronic change affecting splicing. The results confirm previous data by Venturin et al. where the cardiovascular malformations were associated with "NF1 microdeletion syndrome" caused by a deletion of 1.5 Mb in 17q11.2 region.

P0233. Orthomolecular therapy of mitochondrial encephalomyopathy lactic acidosis and stroke-like episode (MELAS): A review of findings and directions for future research

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A recurring problem in the diagnosis and treatment of mitochondrial disorders is the diversity of clinical symptoms that accompany biochemical abnormalities. Well-controlled empirical data with sufficient N are nonexistent. The body of literature with respect to MELAS consists of case reports of varied and inconsistent findings. Currently, there is no treatment for this disorder; however, some researchers have targeted the effects of supplementing patients' diets with enzymes critical for electron transport in mitochondria. This qualitative meta-analytic review synthesizes the findings of 18 case reports both pre- and post-orthomolecular intervention. Most studies did not report specific neuropsychological scores or objective measures of psychiatric symptoms either before or after treatment, emphasizing patients' subjective reports and biochemical findings instead. Generally, patients exhibited depressed IQ with gradual decline in global cognitive functioning. Some patients also exhibited sudden and/or unexplained language deficits, including poor verbal comprehension, decreased word-finding, agraphia, receptive aphasia, phonemic paraphasia, dysarthria and semantic paraphasia. Almost all patients exhibited impaired motor control. Psychiatric symptoms always co-existed with neuropsychological deficits. Patients with defects in the tempoparietal regions evidenced on neuroimaging exhibited impairments in the language domain. Patients with defects in the basal ganglia exhibited psychiatric symptoms. A suggested neuropsychological test battery emerged. Overall, the temporal correlation between patients' clinical improvement and the commencement of treatment with enzymes or co-factors which upregulate oxidative phosphorylation in mitochondria

suggests it may be a useful treatment of patients with MELAS syndrome. These findings may have far-reaching implications as to other disorders of movement and neurodegeneration.

P0234. Del(18p) syndrome with a single central maxillary incisor

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Introduction:

Del (18 p) Syndrome (MIM 607500), which results from the deletion of the short arm of the chromosome 18, was first described by de Grouchy et al. [1963]. There is a broad phenotypic variability associated with the syndrome but the main clinical manifestations are mild to moderate mental retardation, growth deficiency and craniofacial dysmorphism including mild microcephaly, round face, ptosis, epicanthal folds, low nasal bridge, hypertelorism, dysplastic ears, wide mouth with downturned corners, microretrognathia, and dental anomalies. Abnormalities of the limbs, genitalia, brain, eyes, and heart have also been described.

More than 150 cases have since been reported, most of them sporadic, due to "de novo" deletion, although familial cases have been described. About 16% of the cases are due to translocations between chromosome 18 and acrocentric chromosomes.

Clinical Report:

We report a 5-year-old girl that was referred for consultation due to mild mental deficiency, language delay and dysmorphic features including a single central maxillary incisor. Cytogenetics analysis, performed on peripheral blood lymphocytes, by using GTG and CBG banding techniques, showed a translocation between chromosome 13 and 18 resulting in monosomy 18p. Fluorescent in situ hybridization (FISH) studies with chromosome painting probes for the chromosomes involved were performed and confirmed the result. Parent's karyotypes were normal.

Discussion:

Del (18 p) Syndrome is associated with a wide range of clinical manifestations. Although most of the cases are sporadic, family studies are necessary for proper genetic counselling. We emphasize the importance of long follow up of patients affected with del(18p) syndrome.

P0235. Severe myelopathy in brachytelephalangic chondroplasia punctata : a non exceptional complication?

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Brachytelephalangic chondrodysplasia punctata (CPDX1) the RXL form of chondrodysplasia punctata, results from a deficiency in arylsulfatase E. It is usually considered as a minor disorder with mild growth disturbance and facial dysmorphism similar to Binder maxillofacial dysostosis.

We report 4 cases of CPDX1 complicated with severe myelopathy. Two fetuses of a Caucasian woman were suspected for CPDX1 after the discovery of skeletal anomalies by ultrasonographic scan. TOP was done at 26 and 21 WG respectively because of a severe narrowing of the cervical spinal cord observed by MRI in both cases.

In two other unrelated newborns, flat face was noted at prenatal ultrasound scan. CPDX1 was diagnosed neonatally. Both children showed neurological anomalies at birth: severe hypotonia, lack of bulbopontine reflexes and pyramidal signs. Medullary MRI showed vertebral dislocation with cervical spine compression. Both newborns died from neurological complications respectively at day 15 and 25.

Those 4 cases had facial dysmorphism and skeletal anomalies characteristic from CPDX1. Diagnosis was confirmed by the identification of mutations in the ARSE gene for the 3 families.

Life threatening spinal cord complications has not been reported so far in CPDX1. These 4 observations indicate that the prognosis of CPDX1 could be worse than usually reported. Careful, in utero or neonatal MRI imaging of the spine is recommended for fetuses or newborns with Binder face and stippled epiphyses.

P0236. Identification and characterisation of conserved non-coding DNA sequences surrounding the human SOX2 gene

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Microphthalmia, anophthalmia and coloboma (MAC) are among the most common ocular malformations and a significant cause of congenital blindness. Mutations in at least two genes, SOX2 and PAX6 are known to cause anophthalmia in humans. The idea that animal development is controlled by non-coding regulatory elements is now well established in invertebrates. DNA regulatory elements surrounding SOX2 have also been identified and characterised extensively in chicken, with the chick genome believed to represent the best compromise for phylogenetic comparisons with mammals. Using multiple-species alignments we have identified ten conserved non-coding DNA elements surrounding the human SOX2 gene as candidate regions with a role in regulating the expression of the SOX2 gene during development. These 10 sequences that were >95% identical at a nucleotide level across four species (human, mouse, chicken and *Xenopus*), were used for further analysis and had a size range of 80-280bp. To test the hypothesis that mutations in these regions may result in anophthalmia we used DHPLC analysis of PCR products covering these regulatory elements in 66 patients with structural eye defects. We found no evidence that mutations in these non-coding sequences surrounding SOX2 are responsible for structural eye defects in our patient cohort (MAC). This confirms that SNPs are underrepresented in these highly conserved sequences. These regions will now be screened in patient cohorts for deletions and duplications using high resolution CGH microarray analysis.

P0237. Cypher/ZASP gene mutations cause idiopathic dilated cardiomyopathy (IDCM) with poor prognosis.

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IDCM is characterized by ventricular dilatation and systolic dysfunction with signs of heart failure and is inherited in 30-40% of cases. Genetic heterogeneity characterizes familial IDCM. Cypher/ZASP gene encodes a protein that is a component of the Z-line in both skeletal and cardiac muscle. Recent studies demonstrated that Cypher/ZASP knock-out mice develop cardiomyopathy and that defects of the Cypher/ZASP gene may cause familial IDCM. Six major cDNA isoforms of Cypher/ZASP have been identified in human striated muscle and are generated by alternative splicing of a single gene.

We screened the Cypher/ZASP gene in 25 unrelated probands with familial AD-IDCM according to WHO criteria. Levels of serum creatine-phosphokinase were measured to evaluate skeletal muscle involvement.

Three mutations, one novel and two known (Asp117Asn, Ser196Leu and Val588Ile) were identified in three probands (12%) and three related affected relatives. Two of these six patients underwent heart transplantation (a father and his young son), two died [sudden cardiac death (n=1), congestive heart failure (n=1)] while two, from two unrelated families (both young sons of affected patients), are stable in functional NYHA class I and II respectively. None of the patients showed echocardiographic features suggestive for non-compaction left ventricle (NCLV) (Chin et al. and McKenna et al. criteria). We confirm Cypher/ZASP gene as candidate gene for familial IDCM independently on echocardiographic pattern of NCLV. The genetic heterogeneity of familial IDCM is further increasing thus making complex to address patients to the analysis of different disease genes according to the phenotype.

P0238. Mutation screening in DHH (Desert Hedgehog) gene in male infertility

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The DHH (Desert Hedgehog) gene codes for a signalling protein in Sertoli cells, following closely the expression of SRY. Its receptor (PTC1) is localized in Leydig cells and in peritubular cells. Adult and prepubertal Dhh null-mice display abnormal peritubular tissue and

restricted spermatogenesis. Mutations in the DHH gene are associated with 46,XY complete and partial Gonadal Dysgenesis. We present a mutation screening in the DHH gene on 100 male infertile patients affected by different testicular defects and 50 normal controls. 30 patients were affected by non obstructive azoospermia; 45 by severe oligozoospermia (<5million/mL); 25 by moderate oligozoospermia (5-20million/mL). All patients were previously studied for mutations in AR, INSL3 and for microdeletions in Yq. The three DHH exons were amplified by PCR and analyzed by direct sequencing and DHPLC technique.

We detected two polymorphisms in third codon positions in exon 2, not changing codified aminoacid and present in controls: H181H (c>t) and A163A (g>t). We also detected a INV2-3c>a substitution in intron 2 in a patient affected by anorchia. The intronic substitution is carried by the father. This variation was found only once, thus having a frequency of 1/300.

This work evidences that DHH mutations are not a common cause for human male infertility. According to software prediction, the intron 2 substitution could give alternative splicing. Expression studies on mRNA are difficult as the patient lacks testicular tissue, where DHH is expressed. Familiar analysis of patients affected by anorchia does not exclude an hypothesis of imprinting for DHH gene.

P0239. Clinical, cytogenetical and molecular analyses of Sotos and Weaver syndrome in 50 patients

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Sotos syndrome is characterised by pre and post natal overgrowth with advanced bone age, macrocephaly, characteristic facial dysmorphism, and variable mental retardation. Microdeletions of 5q35 region or mutations of NSD1 gene are observed in 60-90% Sotos syndrome and in some patients affected with Weaver syndrome. Furthermore Rio et al. reported 11p15 anomalies in 2 patients with Sotos syndrome. We report on clinical and molecular findings in a cohort of 50 patients with Sotos and Weaver syndrome (age 5 months- 25 years). Large deletions were observed in 5 patients (10%). In 25 patients (50%) we identified NSD1 anomalies. In 20 patients without 5q35 or NSD1 anomalies 11p15 region was studied and was normal. Phenotype-genotype correlations were studied. As previously reported we observed more severe cognitive impairment in patients harbouring large deletions. One patient with a large deletion presented with a severe behavioural phenotype, autoaggressiveness and autistic behaviour. No Renal, cardiac and ophthalmologic malformations were more frequent and more severe in the group with large deletions than in the group with NSD1 alterations. No tumor was observed in our cohort. Advanced bone age and overgrowth were inconstant features. Macrocephaly which was previously considered as a mandatory criterion was not present in 1/30 patients.

P0240. Genotype-phenotype correlations in Larsen syndrome

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Autosomal Dominant Larsen syndrome (LS) is a rare, genetic connective tissue disorder characterized by multiple joint dislocations, distinctive hands and feet, kyphoscoliosis, and a characteristic face. Broad thumbs, cylindrical fingers, short metacarpals, long proximal phalanges, short distal phalanges, and accessory and/or fused carpal and tarsal bones also occur. Similar but more severe features occur in Atelosteogenesis (AO) types I and III. In both LS and AO I/III there are segmentation anomalies of the vertebrae, with the cervical spine being the most severely involved. The typical face in LS reveals midface deficiency with a flat nasal bridge, ocular hypertelorism, and occasional cleft palate. In adults, there may be progressive hearing loss in early adulthood, which is preceded by tinnitus during the late teens. Sequence analysis of FLNB has revealed heterozygosity for

missense mutations in the actin-binding domain of filamin B. These data confirm that dominant forms of LS can result from heterozygosity for missense mutations in FLNB. We have collected clinical materials on 10 genetically independent cases with LS. In 2 of cases, there were independent recurrent mutations that were identical to those found in the original report of FLNB mutations in Larsen syndrome patients. In one family we confirmed germ cell mosaicism in a grandparent with subsequent dominant transmission. We performed a systematic comparison of the clinical characteristics and radiographic manifestations of 120 LS cases reported in the literature, and we compared these features with those in our research subjects.

P0241. Skewed X-chromosome inactivation in the peripheral blood but not in the skin lesion of a female patient with linear scleroderma 'en coup de sabre'

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We describe a 27-year-old woman, diagnosed with scleroderma "en coup de sabre" who has differential patterns of X-chromosome inactivation (XCI) in peripheral blood and atrophic skin lesion cells. The patient presented with linear atrophy of the skin and fat tissue on the right side of her face that began four years before. Thinned epidermis, homogeneous collagen deposition in the dermis and perivascular lymphocytic infiltration was observed in the lesion area. The distribution of the lesion appeared to follow the lines of Blaschko. We recently demonstrated extremely skewed XCI in peripheral blood cells of female scleroderma patients. Based on the observations that frontoparietal or linear forms of the disease follow the lines of Blaschko, we hypothesized that extremely skewed XCI, especially in hematopoietic stem cells, may be involved in disease pathogenesis. Blaschko's lines were originally described as unusual pigmented patterns on the skin, and the boundaries are patches of clonally related X-inactivation derivatives. To test this hypothesis, we analyzed the androgen receptor polymorphism and demonstrate that XCI mosaicism is extremely skewed with the allele ratios of (lower and upper alleles respectively) 96/4 percent in peripheral blood cells. However, XCI patterns were random with 49/51 percent in normal and 35/65 percent in lesion skin biopsy specimens. These results support the hypothesis that extremely skewed XCI could be involved in the pathogenesis of autoimmune diseases including scleroderma.

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P0242. Alström Syndrome: Clinical and Molecular Characterization of two Portuguese families

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Alström Syndrome (MIM #203800) is an autosomal recessive disorder characterized by progressive visual impairment caused by cone-rod retinal dystrophy and early onset obesity. Most patients also develop insulin resistant diabetes mellitus and sensorineural hearing loss during the second decade. Other reported features are short stature, metabolic abnormalities, namely hyperlipidemia, dilated cardiomyopathy, mild to moderate mental retardation, acanthosis nigricans, and chronic renal and liver failure.

Mutations in the ALMS1 gene are detected in 40% of individuals. No other genes have been convincingly associated with this syndrome. We report two unrelated Portuguese patients with Alström Syndrome. Case 1 presented to our clinic at the age of 13, with visual impairment, sensorineural deafness, obesity and acanthosis nigricans. He later developed end-stage renal disease. Case 2 presented at the age of 2 with mild mental retardation, obesity, nystagmus and photophobia. Her mother was already 12 weeks pregnant. Molecular testing of the patients confirmed mutations in the ALMS1 gene in both cases and allowed the prenatal diagnosis of the ongoing pregnancy in Case 2. The clinical presentation of Alström syndrome at different ages is discussed. We also address the issue that this condition probably

remains underdiagnosed. Early diagnosis allows for opportune intervention, particularly early recognition and treatment of diabetes mellitus, nutritional planning, as well as monitoring of heart, liver and kidney function. We also emphasize the importance of early referral of these families for Genetic Counselling and pluridisciplinary management.

P0243. Genotype-Phenotype Correlation in a Deaf Italian Population: an Interdisciplinary Approach

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GJB2 mutations represent the first cause of congenital non syndromic recessive deafness (NSRD).

The 35delG "frameshift" mutation is very frequent in Caucasian population.

Since year 2001 in our Medical Centre a team consisting of audiologists, clinical geneticists and molecular biologists is actively working to improve the etiologic diagnosis and the management of deaf patients. The multidisciplinary approach is relevant to help the patients to understand the meaning of the molecular results, the clinical picture of deafness and the therapies that can be offered.

We analysed audiometric characteristics associated with various types of GJB2 mutations in a population of 289 Italian patients with prelingual hearing impairment to delineate genotype-phenotype correlations. 107 out of 289 patients (37%) presented homozygous 35delG mutation or double heterozygous mutations in GJB2, or the association of a GJB2 mutation with the GJB6 deletion del(GJB6-D13S1830).

Among the inactivating/inactivating genotype mutations 77% (63/81) were associated with profound hearing impairment, 20% with severe and 3% with moderate. For the inactivating/non-inactivating genotype category 73% were associated with profound deafness, 9% with severe and 18% with moderate. The non-inactivating/non inactivating category showed a predominant association with mild deafness (66%). Based on these results we agree with other studies confirming that GJB2 related hearing impairment is mostly determined by the specific mutation combination but there remains phenotypic variability within genotypes.

P0244. Familial Pulmonary Fibrosis in Newfoundland: Evidence for a Novel Genetic Cause

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Idiopathic Pulmonary Fibrosis (IPF) belongs to the group of idiopathic interstitial pneumonias. This disease is limited to the lungs, and is usually an adult-onset disorder diagnosed between 50 and 70 years. The only treatment that improves survival is lung transplant. Without treatment, survival ranges from 2 to 4 years. The literature suggests that up to 3% of IPF patients have a familial form of the disease (familial pulmonary fibrosis or FPF), with at least one other affected first or second degree relative. Autosomal dominant mutations in the Surfactant Protein-C gene (*SP-C*) on chromosome 8p21 have recently been shown to cause familial forms of interstitial lung disease.

In Newfoundland, 11 FPF families containing 35 affected individuals have been identified. DNA has been banked from 250 participants, and cell lines have been established for 234. The study cohort also includes 24 sporadic cases. The medical and family histories of each participant have been reviewed. All participants have had pulmonary function testing and a high resolution CT of chest. The average age of diagnosis in our familial cohort is 55.3 yrs.

The *SP-C* gene has been sequenced from 1 affected individual from each of the families and no mutations were identified. Moreover, immunohistochemical staining of *SP-C* on lung biopsies of 7 FPF patients, all from different families, showed a normal vesicular pattern, suggesting that the families are segregating a novel locus. Linkage simulation studies have been undertaken and have indicated that two families are suitable for genome-wide scans using microsatellite markers.

P0245. Highly tissue specific distribution of the mitochondrial tRNA^{Leu(UUR)} 3243A>G mutation in a patient with deafness, diabetes and severe dementia

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The 3243A>G mutation in the tRNA^{Leu(UUR)} gene of mitochondrial DNA (mtDNA) is associated with a number of different clinical presentations including mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), progressive external ophthalmoplegia (PEO), diabetes and deafness. The routine diagnosis of the 3243A>G mutation in blood is difficult as mutation levels are known to decrease in this tissue over time, while in some patients it may be absent. Although the noninvasive genetic testing of blood samples is common for some mtDNA disorders, patients with the 3243A>G mutation have consistently higher mutation levels in postmitotic muscle than blood, introducing the potential for reporting false-negative results. Here we report the genetic testing of a patient suspicious for a mitochondrial cytopathy. Analysis of the mtDNA extracted from skeletal muscle biopsy specimen did not result in the detection of a mutation. However, postmortem analysis of the mtDNA isolated from brain parenchym revealed the 3243A>G mutation heteroplasmic at a level of 60%. Although muscle and CNS are postmitotic tissues and therefore, supposed to accumulate mtDNA mutations, our findings may be explained by the fact, that embryologically, muscle and CNS are derived from different germ layers. The ectodermal germ layer gives rise to the CNS, and the mesodermal layer to muscular and vascular system. Although, the molecular genetic analysis of skeletal muscle is widely acknowledged to the 'gold standard' in the investigation of patients with suspected mtDNA disease, our findings demonstrate, that molecular testing of muscle mtDNA still has the risk to yield false negative results.

P0246. Modifier genes in cystic fibrosis: frequency of hereditary hemochromatosis in the Hungarian cystic fibrosis population

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Objectives: The variable clinical manifestations of cystic fibrosis (CF) suggest the influence of modifier genes. Gene of hemochromatosis (HFE gene 6p21.3) is a candidate modifier locus for CF based on (1) the suggestion of an association between the HLA loci and CF phenotypes; (2) the location of the HFE gene near the HLA loci and; (3) the similarity between the gastrointestinal manifestations of hereditary hemochromatosis and CF. We have determined the frequency of the C282Y and H63D mutations in a group of 95 CF patients.

Methods: Direct mutation analysis: DNA amplification (PCR) and RFLP analysis were used to test for 2 mutations in the HFE gene. These mutations account for approximately 90% of individuals with hereditary hemochromatosis patients. Patients' clinical statuses were retrieved from the Hungarian CF Registry.

Results: Six patients (3.1%) carried the C282Y and 22 patients (11.5%) the H63D mutations in heterozygous form. Homozygosity for C282Y mutation was not observed, whereas 2 patients carried the H63D mutations in homozygous form. In one case, we were able to detect both mutations (C282Y/H63D). The average gastrointestinal score of CF patients (7.56; n=173) was significantly different from those who carried the HFE gene mutation(s), too (13.9; available n=21).

Conclusion: These data are suggestive of a relationship between the development of gastrointestinal diseases in CF and the HFE gene. Further study of a larger group of patients is warranted.

P0247. Mutations and polymorphisms in CFTR gene in patients with reproductive failure

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Cystic fibrosis is one of the most common serious autosomal recessive inherited disorders in Czech population. The frequency of the disease is around 1/2500 and the carrier frequency is around 1/27.

In pairs with reproductive failures we recommend the analysis of CFTR gene as a preventive examination.

In our laboratory we start with the basic mutation analysis - we examine

five mutations which are frequent in Czech population and in men with severe oligoasthenospermia the polymorphism IVS8polyT.

If a mutation in CFTR gene is found for one of the partners, we analyze a set of 36 CFTR mutations for the other partner.

This analysis detects about 95% of CFTR mutations in Czech population.

2002-2004 we performed CFTR gene analysis in 1325 patients. We detected 55 carriers of one mutation in CFTR gene and two men with two CFTR mutations. In three families we identified both partners as carriers of a CFTR gene mutation. Frequency of CFTR gene mutations in our group is 1/24, 1/41 in women and 1/16 in men respectively.

It is the reason for us to recommend such preventive analysis of CFTR gene in at least one person of the pair with reproductive failure. Most effective is the analysis of men.

In case of families with reproductive failure and with high risk of Cystic fibrosis in offsprings we recommend genetic counselling and prenatal diagnostics or PGD. To people carrying CFTR gene mutations we recommend the analysis of CFTR gene to be done also for their relatives.

P0248. Tackling the diagnosis of sporadic retinitis pigmentosa with a genotyping microarray

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Retinitis Pigmentosa (RP) is a group of inherited and progressive retinal dystrophies that lead to blindness. There are more than 150 loci responsible for these dystrophies. Their genetic characterization becomes even more challenging, because of their additional allelic and clinical heterogeneity. Therefore, searching for the genetic cause of the disease is extremely costly and time consuming, making genetic testing in a clinical setting far from practical, especially in sporadic cases (SRP), which account for 40% of all non-syndromic forms of RP in Spain.

The development of genotyping microarrays, specifically designed to detect all known mutations in every identified gene responsible for RP, will be a major breakthrough for the rapid genetic testing of many uncharacterized patients.

We have started using the LCA microarray, which detects 293 mutations in 8 genes known to cause Leber Congenital Amaurosis, which is a form of severe congenital RP.

A total of 27 SRP patients were genotyped with this microarray (23 with a severe and early-onset form of RP, 3 with moderate RP, and 1 with mild RP).

It detected mutations in 11 individuals (40.7%), of which 43.5% (10 in 23) were severe and early-onset RP, and 33.3% (1 in 3) were moderate RP.

Our results confirm that the vast majority of SRP cases are in fact autosomal recessive.

Although most of these patients are not yet fully characterized, this approach promises to be the tool that could one day become the *de facto* standard in the diagnosis of RP and many other inherited disorders.

P0249. Giant Congenital Melanocytic Nevus: report of two Portuguese cases

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Giant Congenital Melanocytic Nevus (GCMN) is a rare lesion, with an incidence of 1/20000 live births. It is defined as a pigmented lesion, present at birth, corresponding to a benign proliferation of cutaneous melanocytes, measuring 20 cm or more in largest diameter. Lesions can be flat or nodular, with variable pigmentation intensity. Very large lesions are usually localised on the trunk, frequently with smaller satellite lesions on the periphery.

The two main problems associated with this pathology are the risk of developing melanoma, in the nevus itself or in other locations, and the risk of occurrence of Neurocutaneous Melanocytosis (MIM: 249400), characterized by a proliferation of benign or malignant melanocytes in

the central nervous system, which usually carries a bad prognosis. GCMN occurs sporadically, and has a low recurrence risk.

Follow-up and treatment of affected children is aimed at surgical excision or curettage of the lesion to prevent melanoma and early detection of the referred complications. It also provides cosmetic improvement, an important issue for patients and their families.

We report two patients with GCMN presenting at birth, from unrelated families. Both had large pigmented lesions involving mostly the trunk. No other clinical manifestations have been detected so far and cerebral MRI was normal in both. Both were referred to Paediatric Surgery outpatient clinic and keep follow-up in Genetics.

P0250. Genetic counseling of families with t(7;9)(q36.2;p21.2)

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We performed the segregation analysis directly from a relatively large pedigree of five t(7;9)(q36.2;p21.2) carriers in a series of ten pregnancies after ascertainment correction according to Stengel-Rutkowski and Stene. Pedigree was ascertained by a 5 years-old girl karyotyped because of dysmorphic features and distinct developmental profile and monosomy 7q36.2-qter with trisomy 9p21.2-pter (double-segment imbalance) was found. The nature of chromosomal aberration detected using GTG, RBG was confirmed by multicolor chromosomal analysis, utilizing the commercially available M-FISH KIT (MetaSystem GmgH, Altlussheim, Germany). The slide treatment, post hybridization washes and detection were performed according to the manufacturer's protocol. The image analysis was done using an epifluorescence microscope (Leica DMRB, Wetzlar, Germany) and image analysis software (QUIPS, Vysis, IL, USA). A study of morphological phenotype of proband according to uniform protocol of clinical and anthropological traits taken from Munich Database elaborated by Stengel-Rutkowski. Quantitative features analysis showed that a total of 125 out of 807 (15,66%) of catalogued anamnestic and morphological traits was present in our child. Morphological phenotype of our patient compared to quantitative definition of monosomy 7q36.2-qter and to definition for trisomy 9p21-pter separately showed 24 of 125 (19%) features and 61 of 125 (48%) features respectively. The individual probability assessment for unbalanced progeny at birth after 2:2 disjunction and adjacent-1 segregation were 30±14.5% (high) and 10±9.5% for miscarriages and similar for stillbirth/early death. These values of probability of occurrence of unbalanced progeny at birth and other unfavorable pregnancy outcomes may be used for individual genetic counseling of carriers with t(7;9)(q36.2;p21.2).

P0251. Mutations in CDKL5/STK9 are associated with X-linked infantile spasms and a severe variant of Rett syndrome

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Recently, we showed that truncation of the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene, also known as serine threonine kinase 9 (STK9), caused mental retardation and severe neurological symptoms in two unrelated female patients with balanced X;autosome translocations. CDKL5 is subject to X-inactivation in normal female somatic cells, and is functionally absent in the two patients due to preferential inactivation of the normal X.

Subsequent screening of a small panel of patients who had been diagnosed with Rett syndrome (RTT) or a variant of RTT and no identified pathogenic mutation in MECP2, led to the finding of two additional mutations in CDKL5: a cysteine to phenylalanine amino

acid exchange in one patient presenting with early-onset seizures, psychomotor development delay and absence of speech, and an arginine to serine exchange in a monozygotic twin pair who exhibit almost identical clinical features of classic RTT, with a history of early-onset seizures. Both mutations most likely occurred *de novo* and affect highly conserved amino acids which are located within a predicted protein kinase domain. Our results strongly suggest that impaired CDKL5 catalytic activity plays an important role in the pathogenesis of this neurodevelopmental disorder. Further studies aim to elucidate the specific mechanisms by which CDKL5 alterations lead to impaired development.

P0252. Midline Anomalies in a Boy With Transverse Limb Defects: A New Syndrome or Adams-Oliver Variant?

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This one-year old boy was seen in genetics clinic because of multiple congenital anomalies. He was the product of a non-consanguineous union of an Italian couple, after a non-eventful pregnancy, without maternal exposures to teratogens. He was delivered full term and discharged home within 2 days. At birth, he was noted to have transverse limb defects involving all fingers and toes. Hypospadias was also noted. He subsequently developed feeding issues and was noted to be hypotonic. Cranial imaging detected partial agenesis of the corpus callosum, abnormal brain stem configuration, a cleft in superior cerebella vermis and abnormal cerebella grey-white matter junction. The family history is non-contributory. On genetics assessment, he has relative macrocephaly with prominent occiput, with sparse scalp hair, eyebrows and eyelashes. He has low-set ears and carp shaped mouth. The fingers end abruptly, as in amniotic band sequence, without visible fingernails. The shoulders are sloping and narrow with dimples. The toes appeared tapered with rudimentary toenails. There was minimal 2-3 syndactyly of the toes. The length of the limbs was normal. The external genitalia were male with hypospadias, chordee and asymmetric foreskin with high insertion of the scrotum. Neurologically, he is hypotonic with severe global delay. His karyotype was of normal male, 46,XY. 7-DH and metabolic workup were also within normal limits. Genetic testing for Filamin A gene, responsible for oto-palato-digital syndrome types I and II as well as grey matter heterotopia, is underway. We are also considering subtelomeric FISH. Overall, this appears to be a new syndrome.

P0253. Deformities of the skull in children: clinical markers of the polymalformative syndromes

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Background: Craniostenosis is defined as a premature closure of cranial sutures. The incidence of primary craniostenosis approximates 1 per 2,000 births. The cause may be chromosomal abnormalities, genetic anomalies or multifactorial. Most cases of craniostenosis are evident at birth.

Materials and methods: Four cases with congenital deformities of the skull are presented, two males and two females, aged 3-18 months.

Results: By clinical examination, X-ray films and CT scans of the skull was found scaphocephaly in two cases, turricephaly in one case and right parieto-occipital plagioccephaly in one case. Cortical atrophy and mental retardation were present in all cases. Optic atrophy and seizures were found in two cases. Facial dysmorphism and kidney defects were present in two cases. Heart defects were associated in one case. Just in one case craniotomy was performed.

Conclusions: In 50% of cases the diagnosis was made after 1 year of age and just 25% of cases were operated. Without an early diagnosis and surgical correction of the deformities the growth of skull is inhibited and brain is seriously damaged.

P0254. An eleven year old girl from Northern Lithuania with confirmed early-onset torsion dystonia

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Our patient is an eleven-year-old girl from the third non-complicated pregnancy of her mother. She suffers from torsion dystonia. The genealogy of this family is complicated: the girl's grandfather and grandmother have suspected Parkinson's disease; her grandmother's brother has the same features as our patient. The onset of the disease started at age eight with splay gait. Two years after the first focal manifestations the girl started to complain of writer's cramp. The patient was evaluated using laboratory and instrumental investigations: ceruloplasmin, serum and urine organic acids and amino acids were without pathological changes. MRI of the brain was normal. Electromyography showed signs of denervation in both feet, but it was more expressed in the right. A heterozygous deletion 904-906GAGdel was detected in the *DYT1* gene (in collaboration with Dr. F.A. Hol, Nijmegen). This result confirms the diagnosis of early onset torsion dystonia caused by a mutation in the *DYT1* gene. The model of inheritance for early-onset generalized dystonia is autosomal dominant. But only 30 to 40% of those with a mutated *DYT1* gene develop symptoms of dystonia. Due to this variable penetrance about 60 to 70% of people who carry the mutated gene will not manifest symptoms.

P0255. A New X-Linked Craniofacioskeletal Syndrome with Female Expression and Male Lethality

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A syndrome with multisystem manifestations has been observed in three generations of a Caucasian family. The findings in six females provide a composite clinical picture of microcephaly, short stature, small retroverted ears, hypotelorism, full tip of the nose overhanging the columella, short philtrum, thin upper lip, soft tissue excrescences at the angle of the mouth, small mandible, small hands and feet with brachydactyly, finger V clinodactyly, flat feet, an excessive number of fingerprint arches, and mild impairment of cognitive function. Two males were more severely affected and died in the initial months of life. They showed intrauterine growth retardation, broad cranium with wide sutures and fontanelles, cardiac defects, small hands and feet with abnormal digital creases and small nails, and genital abnormalities. Chromosome analysis, FISH for 22q11 deletion, plasma cholesterol and 7-dehydrocholesterol levels have been normal. MRI studies of 4 of the females showed reduced total brain volume compared to age-matched controls without a reciprocal increase in CSF. This suggests a defect in an early neurodevelopmental process. X-inactivation studies show near complete skewing in two affected females, but were not informative in three others.

The skewing of X-inactivation, the different severity in males/females and some overlap with OPD-II, suggested *FLNA* as a potential candidate gene. However, no pathogenic alteration was identified. Based on clinical presentation and the X inactivation data, we believe this family represents a new X-linked mental retardation (XLMR) syndrome and is a new member of the group of remarkable X-linked syndromes which occur predominately among females.

P0256. Molecular diagnostics of acute intermittent porphyria in Russia

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Acute intermittent porphyria (AIP) is an autosomal dominant inherited disorder characterized by a deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthetic pathway. Clinically, AIP is characterized as acute neurovisceral attacks that are often precipitated by exogenous and endogenous factors such as hormones, drugs, alcohol and others. To date, at least 250 different mutations causing AIP are known.

We inspected 46 unrelated Russian patients affected by AIP. Mutational analysis was performed by direct sequencing using PCR or RT-PCR gene or cDNA PBGD fragments, containing all functionally important gene regions, including exons, exon-intron junctions, promoter and poly(A)-signal. We identified 34 different mutations including 22 novel,

which have not been observed in world literature before. Found mutations represent 15 splicing mutations, 10 missense mutations, 5 deletions, 3 nonsense mutations and one small insertion. 53delT (5 cases) and Arg173Trp (5 cases) were the most frequent mutations among our patients. Family-specific mutations were screened among relatives of 25 studied patients by restriction heteroduplex or sequencing analysis. Among 85 studied individuals 31 were asymptomatic carriers of AIP. Early diagnostics for mutations of PBGD gene allow to decrease the number of AIP cases which lead to severe complications, physical inability and lethal outcomes.

P0257. An inherited inversion of chromosome 6 and its clinical phenotype

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This is the report on a family with two out of three siblings and their father presenting with the same neurological phenotype and an inherited chromosomal aberration.

The young daughter of the family sought genetic counselling as she was concerned that the tremor and shaking that she had was inherited taking into account the fact that her father and brother also had similar symptoms.

The proband, her brother and father presented with involuntary movements, abnormal body posture, dysarthria and squint. There was history of learning difficulties in the father and brother and evidence that they had mild mental retardation. The proband had no history of significant learning difficulties.

Chromosomal analysis revealed an abnormal karyotype with an inversion of chromosome 6p23q12 in all three patients.

The clinical phenotype, full investigations family and medical history (including data on their general health and other aspects of their life) are discussed in this report.

P0258. Inherited ulcero-mutilating neuropathies CMT2B and HSN1 in Czech families.

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Inherited ulcero-mutilating neuropathies are rare axonal polyneuropathies with prominent sensory loss, foot ulcerations and usually autosomal dominant inheritance.

Mutations in the RAB7 gene cause CMT2B neuropathy and mutations in the SPTLC1 gene cause HSN1. Only few such families were reported so far worldwide and no such cases have been reported from Czech Republic.

We describe two Czech families one with CMT2B and one with HSN1. In the CMT2B four-generation family we studied two patients from two generations. The disease presented by painless foot injuries and prolonged and difficult healing in the middle of the second decade. Motor deficit presented with distal muscular weakness of legs in the third or fourth decade. Legs were earlier and more affected than hands. Tendon reflexes were absent in one patient and well preserved in the other. Electrophysiology showed prominent axonal polyneuropathy more pronounced in the legs and mild in arms. A previously reported mutation L129F in the RAB7 gene was detected in both patients.

In the HSN1 four-generation family we studied eight affected patients from three generations. The age of onset was in the third to fifth decade. Two persons underwent amputations in the legs in the sixth decade. All affected patients showed prominent sensory loss predominantly in temperature and pain and only mild in vibration. EMG showed axonal neuropathy with unexcitable nerves distally in the legs. Some patients presented increased patellar tendon reflexes. A previously reported mutation C133Y was detected in all eight affected patients of this family.

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P0259. Particular features of the Congenital Heart Defect in Children over more than a decade

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The aim of the study is to present the evolution of the congenital heart defects(CHD) in children from Timisoara, over a period of 11 years, time in which we felt the consequences of the nuclear accident in Chernobyl-Russia and the war in Yugoslavia, country nearby. The study was done on a cohort of 1113 children with CHD, classified in two groups: 226 children between 1990-1994 and 887 children between 1995-2001. The number of CHD between first and second group arise 3,92 times; the noncyanotic CHD arise 4 times and the cyanotic CHD arise 3 times. From the noncyanotic CHD, left to right shunts arise 3,5 times, complex malformations arise 7,5 times and obstructive CHD 5,4 times. In the first group, 90,7% were noncyanotic, 9,3% were cyanotic. The noncyanotic CHD were composed of: 83,40% left to right shunt, 8,30% complex CHD and 8,30% obstructive CHD. From the left to right shunt malformations, the dominant were: ASD (55,12%), VSD (20,48%) and PDA (7,8%). In the second group, 92, 67% were noncyanotic and 7,32% were cyanotic. The noncyanotic CHD were composed of: 72,99%, left to right shunt, 15,69% complex CHD and 11,32% obstructive CHD. The dominant left to right shunts were: ASD (40,75%), VSD (19,82%), and PDA (12,40%). The number of CHD arise 3,9 times in the second period vs the first one, most of the cases registered between 1997-2000. The noncyanotic CHD were dominant in both groups. The most important rising in complex CHD were in the second group, as a result of the teratogenic factors to which Romania was exposed.

P0260. Prognostic importance of complex abnormalities of caryotype in patients with myelodysplastic syndrome

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Determination of caryotype in patients with myelodysplastic syndromes (MDS) during any leukemic transformation and cancer progression is an important prognostic factor. Intensification of MDS treatment is connected with a diversity of complications, therefore determination of prognostic risk groups becomes more and more actual goal. It is well known that chromosome aberrations are distinguished in 60% of initially diagnosed patients with MDS approximately, complex abnormalities are observed in 10-30% of patients.

150 patients with MDS were examined, complex abberations were observed in 21 patients (14%), age median of them was 55 years. Cytogenetic analysis revealed quantitative abnormalities of caryotype in 17 patients (81%) from 21 (17/21). Changes affected chromosomes 5, 7, 8, 10 and 21. Among structural anomalies, observed in all patients, abberations of chromosome 1 - 4/21 (19%), del5q 4/21 (19%), del7q 6/21 (28,5%), different abberations of 12p13: t(3;12), del12(p13) and add12(p13) 3/21 (14%) - were found more often. Anomalies of 8q - del8(q13), t(8;17)(q24.1;p13) and t(8;18)(q24;q23) were observed in 4 patients from 21 (19%). Such rare abberations as dic(1;11)(p36;p15), t(11;22)(p14;p10), dic(5;11)(p15;q25), t(2;5)(p24;q23), t(16;17)(q24;q12), der(14;14)(q10;q10) and der(13;14)(q10;10).

An application of immunosuppressive therapy by cyclosporine A and standard schemes of PCT (polychemotherapy) was uneffective in 19 patients (90%) from 21, 16 patients (76%) have died. Obtained data point on very negative clinical course of MDS with complex abnormalities of caryotype that is probably caused by instability of genome and particular biological features of cells of patients with MDS.

P0261. Study of the phenotype of unrelated patients (males and females) with premutation and intermediate alleles of FMR1.

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The physical characteristics and behavioural phenotype of full mutation patients with fragile X syndrome are well established, while carriers of premutations and intermediate alleles of FXS are traditionally regarded

as being clinically unaffected.

Recent reports suggest that carriers of premutation and intermediates alleles may show phenotypical features: physical, psychological, speech-language problems, premature ovarian failure (POF) in women and a neurodegenerative disorder, fragile X associated tremor/ataxia syndrome (FXTAS) in older adult carriers.

Patients attending the Neurological and Gynecological clinics were evaluated and when FXTAS was suspected, they were sent for molecular analysis. Informed written consent was given by the families.

Southern blot analysis and PCR were carried out to establish the exact number of CGG repeats.

We have found 14 premutation (12 females and 2 males) and 7 intermediate alleles (5 males and 2 females). Protein expression was studied in all, and it was within the normal range.

In this study of unrelated patients with premutation and intermediate alleles, males and females (ages between 3 -12 years old) showed similar features: language impairment in concentration skills and /or distractibility, attention deficit, anxiety, communication disturbance, speech and language problems (difficulty with generating and ordering words in sentences) and all presented delayed early language acquisition.

Despite the limitations of the study, our findings suggest that CGG repeat lengths do not permit predictions about clinical features in patients with premutation and intermediate alleles, and therefore other studies must be established.

P0262. Molecular analysis of SMN gene of SMA Tunisian patients

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Introduction: With a prevalence of 1/6000 to 1/10000, Spinal muscular atrophy (SMA) represents the second most common fatal Autosomal recessive disorder after cystic fibrosis. SMA is characterised by degeneration of the anterior horn cells of the spinal cord resulting in progressive symmetric proximal muscular weakness.

In this study we report molecular screening of 5 patients clinically diagnosed as SMA.

Material and method: The most commonly used method to confirm genetically the diagnosis of SMA is a qualitative PCR-RFLP assay to detect the homozygous absence of SMN1 (Survival Motor Neurone). Two highly homologous survival motor neurone genes, SMN1 and SMN2, are present at the same locus. The detection of the homozygous deletion of exon 7 of SMN1 gene, which is present in 90% to 98% of the patients, takes advantage of the base difference in this exon to distinguish SMN1 from SMN2.

Results: Within those 5 patients, 4 are carriers of a homologous deletion of SMN1.

Only one, despite clinical diagnosis of SMA, doesn't show any deletion.

Discussion: This qualitative method can be used to determine whether an individual lacks both copies of SMN1 exon 7, but can not distinguish carriers with one copy of SMN1 from normal individuals with two copies of SMN1. To identify SMA carriers necessitates a quantitative approach.

Conclusion: The clinical diagnosis of SMA can be problematic, therefore genetic testing is ordered to identify patients and deliver a directed genetic council.

P0263. Clinical phenotype of a boy with karyotype 46,XY,t(11;22)(q23.3;q11.2)

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We report on a boy with rare set of signs and abnormal karyotype. For the first time we examined the proband aged 13 yr. Initial diagnosis was hypogonadism, hypothalamic dysfunction, neurovegetative dystonia (hypertensive type), obesity (moderate type), macrosomia (disharmonic type). Proband was born after the second gestation prolonged to 28-yr-old healthy female by spontaneous vaginal breech labor. The first pregnancy ended in spontaneous abortion. Proband's father

suffered from oligozoospermia. Acute cardiovascular insufficiency caused exitus lethalis of proband's father aged 40yr. His parents were not consanguineous. At birth proband weight was 3100g, his length was 51cm, head circumference was 35cm, chest circumference was 34cm. At the age of 13 years his length was 162cm, his weight was 80900g, testes 2ml and 2.5ml, penis 4.5x1.8cm. Test with human chorionic gonadotropin was positive. Attacks of severe headache (fronto-parieto-temporal areas), blood hypertension (150/100; 160/120 mmHg), nasal and ear haemorrhages were the symptoms of his disease. Decreased levels of LG and testosterone were shown. Osteoporosis of hand more severe in distal parts, structure disturbance of radius were observed with X-ray investigation. Proband karyotype was found to be 46,XY,t(11;22)(q23.3;q11.2). This point of breakage is situated closely to Bernard-Soulier syndrome (BSS, MIM231200). Authors assume relationship between some clinical features like BSS and nature of chromosomal aberration.

P0264. Clinical and molecular findings in 41 Belgian patients with Pseudoxanthoma Elasticum

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Pseudoxanthoma Elasticum (PXE - MIM#264800) is a hereditary connective tissue disorder comprising cutaneous, ophthalmological and cardiovascular manifestations. It is caused by mutations in the ABCC6 gene (31 exons) located on chromosome 16p13.1, encoding a transmembrane transport protein of which the function and substrate are presently unknown.

We present phenotypical and molecular data on 41 patients with biopsy proven PXE.

Great variability in severity of the cutaneous lesions was observed, while ocular involvement was consistently present. A significantly higher incidence of peripheral vascular complications and stroke were found, whereas coronary and valvular symptoms were much less common than suggested in the literature.

Mutation analysis of the ABCC6 gene identified at least one mutation in all but one patient (98%), with R1141X being the most prevalent. Forty-eight percent of mutations were observed in exon 24, and 22% were localised in exons 29, 28 and 18.

Patients homozygous for R1141X (exon 24) had a significantly higher incidence of peripheral vascular disease than those with at least one N-terminal mutation. This difference might be explained by the observation that N-terminal exons do not code for the important Nucleotide Binding Folds.

We conclude that exhaustive clinical examination prior to mutation analysis results in a high mutation detection rate in this variable disease. Since analysis of 4 ABCC6 exons identifies 70% of all mutations, a two-step method can be used, analysing these four prior to the rest of the gene.

P0265. Genetic factors modifying CADASIL phenotype?

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited systemic vascular disorder causing recurrent brain infarcts and leading to subcortical vascular dementia. The onset, duration and clinical symptoms of the disease are highly variable. The defective gene is NOTCH3 and over 100 different pathogenic mutations have been identified. In Finland (population 5 millions) we have so far diagnosed 21 CADASIL families with over 100 patients sharing the same NOTCH3 mutation, R133C. Despite this uniform mutational background the phenotypes vary significantly. This indicates additional genetic or environmental factors modify the phenotype.

We selected two groups of patients having the same mutation (R133C): one group of patients with early onset and severe symptoms and another group with late onset and mild or no symptoms.

Seven *intragenic NOTCH3 polymorphisms* causing a change in the amino acid were tested in both groups. None of the polymorphisms

correlated with the phenotype and therefore do not affect the clinical variation.

Apolipoprotein E allele ε4 is a known risk factor in Alzheimer's disease. In several studies it is also suggested to have an effect on vascular dementia. Thus our two patient groups were genotyped for *APOE* gene. Results of this analysis will be presented.

P0266. New polymorphic microsatellites provide a quick, cheap and reliable method to detect common and atypical duplications and deletions of the 22q11.2 region

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Low copy repeats (LCRs) are the cause of most duplications and deletions in the 22q11.2 region, causing the 22q11.2 duplication and deletion syndromes. The del22q11.2 syndrome is the most frequent genomic syndrome with an incidence of 1/4000 births. The most frequent deletion has a size of 3 Mb and is flanked by two LCRs (LCR22-2 and LCR22-4). However, less frequent atypical deletions and duplications are also in most cases flanked by other LCR22s of the same region (LCR22-3, LCR22-5 and LCR22-6). There is a total of 5 intervals flanked by LCRs spanning approximately 6 Mb. We have developed a strategy that provides a reliable, quick and cheap method to detect deletions and duplications caused by these LCRs. This is based on the typing patient's DNA extracted from blood or uncultivated amniocytes with a panel of 9 highly polymorphic microsatellite markers that are distributed over the 5 intervals that could be duplicated or deleted by LCR-mediated illegitimate recombination. Heterozygous microsatellites demonstrate the absence of a deletion or a duplication (that would show 3 alleles in most cases). With this first screening we are able to rule out deletions and duplications in over 85 % of patients. If microsatellites are non-informative within one LCR-flanked region, microsatellites of a second panel are typed. Intervals with no informative microsatellites will be suspect of harbouring a deletion and further analysis will be required (linkage analysis with parental DNA or MLPA or FISH).

P0267. Craniofacial anomalies associated to camptodactyly and chest deformity : Teebi-Shaltout syndrome or new entity.

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We report the case of a Tunisian boy with unusual face, marked flat occiput, camptodactyly, chest deformity, normal thrive and normal intelligence. He was born to consanguineous parents.

He had brachycephaly with marked flat occiput, flat supraorbital ridges, hypertelorism, epicanthal fold and bilateral ptosis. The palpebral fissures were short and downslanting. He had depressed nasal bridge, broad nasal tip with wide nostrils. The philtrum was long with thin upper lip. He had maxillary hypoplasia, high and narrow palate, oligodontia and dental malocclusion. His ears were stickled with severe lobule hypoplasia. The hair was normal and his neck was short. He had thorax asymmetry, pectus excavatum and depressed low sternum. He presented moderate restrictive lung disease. We noted bilateral camptodactyly of all fingers, and multiple cutaneous nevi. There was no other abnormality. Neurological examination was normal, and there was no clinical features of visceral malformation.

He had normal psychomotor evolution and intelligence was normal. A skeletal survey was unremarkable except skull roentgenography showing deformity. The karyotype was 46 XY. A part form ptosis, ophthalmologic examination was normal. There was not hearing loss. The mother presented moderate left ptosis, unilateral left deafness and she had abnormal teeth position but normal teeth number. His grand parents, uncles and aunts were phenotypically normal.

This phenotype may match with Teebi-Shaltout syndrome previously described, but some features are lacking such as hypotrichosis, hypohidrosis, frontal bossing, prominent occiput.

Does this observation corresponds to Teebi-Shaltout syndrome or a new entity.

P0268. Tumor necrosis factor β gene polymorphism in Myasthenia Gravis patients from Bashkortostan, Russia.

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Myasthenia Gravis (MG) is an autoimmune disorder mediated by autoantibodies against the nicotinic acetylcholine receptor (nAChR) of neuromuscular junction. The genetics of the autoimmune response in MG are not well understood, although different polymorphic sets of genes are currently under study. TNFB is the candidate gene as it encodes cytokine TNFβ that play an important role in the regulation of the immune response through the induction of genes such as MHC genes and the activation of immune cells. The aim of our study was to analyze the association between alleles of the TNFB Ncol restriction fragment length polymorphism with MG and the disease subgroups in 98 MG patients and 78 healthy controls. Comparison of phenotype, allele and genotype frequencies of TNFB polymorphism did not reveal any significant difference between patients as a whole and controls. Allele frequencies of TNFB gene polymorphism in patients and in healthy controls for allele TNFB*1 were found to be 30,7% and 30,8%, correspondingly. However, when patients were subgrouped according to clinical forms of the disease, the frequency of the TNFB*1 allele was found to be statistically significant decreased in patients with the ocular form (4,5%) compared to patients with the generalized form (35,0%; p=0,0052) and healthy control (30,8%; p=0,0091). No TNFB*1/1 genotype were observed in patients with ocular MG. Thus, allele TNFB*1 and genotype TNFB*1/1 are negatively associated with the ocular form of the disease. Our results confirm the hypothesis that the MG is a heterogeneous disease with the complicated pathogenic mechanisms.

P0269. Laurence-Moon-Bardet-Biedl syndrome- case presentation

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Laurence-Moon-Bardet-Biedl syndrome has as principal abnormalities: obesity, mental deficiency, polydactyly and/or syndactyly, retinitis pigmentosa, genital hypoplasia or both, and has an autosomal recessive transmission. We present the case of a 16 year old boy, who was admitted to our clinic because of cyanosis, especially on effort, dyspnea and fatigue. On clinical examination, we noted an obese child, grade III, 82 kg, with cyanotic congenital heart defect, Fallot disease, oxygen saturation 64%, polydactyly (of the 1st metacarpal and phalanges) of the left hand, and radio-cubital synostosis of the same hand, with reduction in pronation-supination movement, moderate mental deficiency (IQ 69), anterior hypospadias, bilateral cryptorchidism, hypoplastic scrotum, micropenis with hypogonadism, and hirsutism. The insulin level was normal; FSH and LH hormone were much than normal, and the testosterone levels corresponded with Tanner Stage 4. The boy did not have retinitis pigmentosa, only myopic astigmatism. We considered the possibility of a diagnosis of Holt Oram syndrome, but all the associated abnormalities, except the skeletal and cardiovascular ones, are found mainly in Laurence-Moon-Bardet-Biedl syndrome. In conclusion, complex abnormalities needs complex investigations and collaboration between the specialities, under the cover of the genetic consultation, early in childhood, to prevent and correct problems as far as possible.

P0270. Homozygous M34T mutation of GJB2 (CX26) gene associates with a recessive nonsyndromic prelingual hearing loss in several families with a clinical finding of a 2 kHz impairment in the audiogramm

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Mutations in the gap junction beta 2 (GJB2) gene are the most common cause of congenital nonsyndromic hearing impairment (HI). More than 80 GJB2 mutations have been reported and one specific mutation, 35delG, accounts for 60% - 70% of the cases. The

controversial allele variant 101T>C (M34T) has been proposed to cause autosomal dominant or recessive nonsyndromic HI or act as a nonpathogenic polymorphism with no clinical significance.

In our routine GJB2 mutation screening of patients with HI performed by direct sequencing, we found thirteen patients homozygous for the M34T mutation in northern and eastern Finland. They came from seven families with prelingual nonsyndromic sensorineural HI. In five families, where the phenotype of the M34T could be followed from all the affected and healthy family members, the HI segregated with the genotype M34T/M34T.

We have analyzed the audiological data from the six families both from the affected persons and the family members. Out of the twelve children homozygotes for the mutation M34T, there was a significant finding of a 2 kHz impairment of moderate to severe degree in nine of them and in two child the impairment was at 3 kHz. In one child the impairment was not as clear.

Our findings give evidence that the M34T mutation is clinically significant showing recessive mode of inheritance. Furthermore, a specific shape of the audiogram with an impairment at 2 kHz was associated with homozygous M34T mutation in the families studied.

P0271. A new case of 3M syndrome

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Three M syndrome is an extremely rare syndrome. About fifty cases are reported in the literature. The syndrome was first described in 1975 by Miller and al. Common clinical features are : low birth weight, short stature (dwarfism), craniofacial malformations, bone abnormalities, and hypogenitalism. The syndrome is thought to be inherited as an autosomal recessive genetic trait. The differential diagnosis with other dysmorphic dwarfisms such as the Silver-Russel syndrome, the Mulibrey dwarfism and the fetal alcohol syndrome . The cause and the molecular basis of this syndrome are still unknown.

We report the case of a 9 years old dwarf Tunisian male. He was born from consanguineous parents, both have short stature. He was very small at birth, and he had characteristic craniofacial features with dolichocephaly, triangular-shaped face, pointed chin, high forehead, anti-mongoloïd palpebral fissures, long philtrum, prominent lips, high narrow palate, and large ears. He had a short neck, pectus excavatum, lumbar hyperlordosis, clinodactyly of the 5th fingers, joints hyperextensibility, genu valgum, and flat feet. He had a hypogenitalism and normal intelligence. Radiographs showed a spina bifida L4-L5, the vertebral bodies were tall, with a wide spinal canal; congenital dislocation of the hip, and a delayed bone age were noted. The hormonal investigation (GH, IGF1, TSH, FT4) showed normal levels. The karyotype was 46, XY. Treatment with growth hormone is attempted in order to allow the child an eventual increase of his final size. The inheritance pattern and of the pathogenesis of this syndrome are discussed.

P0272. Evaluation of clinical and cytogenetical criteria presets for the diagnostic approach in a large group of Fanconi anemia cases

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Fanconi anemia (FA) was originally described as an autosomal disorder characterized by a progressive pancytopenia, diverse congenital abnormalities and increased predisposition to malignancy. Growth retardation, abnormal skin pigmentation and thumb anomalies are the typical manifestations along with some rather rare morphometric and systemic abnormalities found in FA patients. While neither of these clinical presets are adequate, cytogenetic analysis should always be carried out following the clinical stigmata and be acknowledged for the diagnosis of the disease. The current method of choice in cytogenetic analysis depends on the comparison of spontaneous versus diepoxybutane (DEB) induced chromosomal breakage for diagnosis. Here we present a data accumulated during last 15 years from the patients referred to our medical genetic department with FA prediagnoses. Out of 181 cases 103 were confirmed as FA after DEB induced analysis. Multivariate logistic regression was utilized for modelling the effects of clinical and cytogenetical findings and revealed

clearcut predictors between FA and non-FA groups.

P0273. Acromicric Dysplasia versus Geleophysic Dysplasia: report of a portuguese patient

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We report a girl, fourth child of consanguineous parents in fourth degree, with normal stature, and no relevant family history. She was referred to us at 6 months age because of severe prenatal and postnatal short stature, peculiar face with narrow palpebral fissures, long eyelashes, short and wide nose, small and stubby hands and feet with very short distal phalanges with redundant skin. She had mild dorsal kyphosis, short neck and thorax and joint limitation. The mitral valve had a subvalvular anomaly and a L-R shunt, which required surgery. Mild squint and hypermetropia was diagnosed. Neurological examination was normal. X-ray study showed severe carpal bone age delay, very short and coarse second and third phalanges, femoral heads looked normal. No other anomalies were detected.

She is now 8 years old and shows severe short stature, contractures are still evident, hands and feet remain very short and stubby, facial appearance is very appealing, there is no hepatomegaly, cardiac status remains stable. She is very shy and has a few learning problems. All other investigations were normal (brain MRI, EEG, abdominal US, immunological and endocrine tests, karyotype, LCFA, GAGs, metabolic screen).

Although the phenotype could be compatible with Geleophysic Dysplasia and the parental consanguinity supports this, the lack of evidence of accumulation features leads us to classify this patient as Acromicric Dysplasia. Once more, this case stresses the progressively accepted phenotypic overlap between these two diseases.

P0274. Spondyloepimetaphyseal dysplasia associated with joint laxity and multiple dislocations, mental retardation, retinopathy and deafness

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The term spondyloepimetaphyseal dysplasia (SEMD) embraces a large group of disorders, distinguished on the basis of clinical and radiological features.

We report the first and only son of a consanguineous couple (first cousins), referred to us at 33 months of age for confirmation of bone dysplasia. He showed severe short stature of prenatal onset, severe kypho-scoliosis and a skeleton x-ray compatible with a spondyloepimetaphyseal dysplasia. He also presented with congenital hip dislocation, joint laxity and multiple dislocations. Large eyes with blue sclera, and short neck were noted, but no real facial dysmorphism. Mild mental retardation and bilateral sensorineural deafness were first noted at age 5. He also had astigmatism, mild optic atrophy and retinitis pigmentosa diagnosed at age 6.

All the investigations performed thus far were negative (karyotype, metabolic screen, LCFA, lisosomal disorders screening, brain MRI, abdominal US and echocardiogram).

The father has a similar face appearance, joint laxity with multiple dislocations, but no short stature or other anomalies.

It is very difficult to establish a clear mode of inheritance in this family, since SEMD with multiple dislocations has an autosomal dominant pattern and SEMD with joint laxity an autosomal recessive one. On the other hand, the association MR, retinopathy and deafness lead to mandatory exclusion of a mitochondrial disorder in spite of consanguinity.

We believe this patient may represent a unique form of spondyloepimetaphyseal dysplasia associated with other anomalies. To our knowledge, the only similar previously reported patient was also of Portuguese origin (Liberfarb RM et al., 1986).

P0275. Floating Harbor syndrome : report of 6 new cases**s. sigaudy, c. missirian, n. philip, a. moncla;**

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Floating Harbor syndrome is a rare genetic disorder. Only about thirty patients have been reported since first description in seventies. To date, genetic bases are unknown. Clinical diagnostic is based on characteristic facial dysmorphism including triangular face, deep set eyes, prominent nose, large columella, short philtrum and thin lips, proportionate short stature with delayed bone age and expressive language delay. Most cases are sporadic but two patients with affected parents have been reported suggesting an autosomal dominant mode of inheritance. One case of recurrence in sibs could be due to gonadal mosaicism. We report 6 new patients aged from 4 to 31 years. Four were sporadic cases and there was a familial transmission from father to daughter. All displayed typical facial dysmorphism and short stature. In addition, two patients had congenital cardiac defects (pulmonary stenosis and atrial septal defect), two boys presented hypospadias and two girls developed epilepsy. As psychomotor development is normal or mildly retarded and associated malformations not usual, patients are generally referred to paediatricians because of growth retardation

P0276. Autosomal dominant perniosis maps to chromosome 3p**M. Linné¹, M. Gong², H. Schulz², M. Meurer³, E. Schröck¹, M. Gahr⁴, N. Hübner², M. A. Lee-Kirsch¹;**¹Institut für Klinische Genetik, Technische Universität Dresden, Dresden, Germany, ²Max-Delbrück-Center for Molecular Medicine, Berlin-Buch, Germany,³Klinik für Dermatologie, Technische Universität Dresden, Dresden, Germany,⁴Klinik für Kinder- und Jugendmedizin, Technische Universität Dresden, Dresden, Germany.

Familial perniosis is a novel autosomal-dominant genodermatosis. The clinical picture consists of painful purple-red inflammatory lesions in acral locations such as fingers, toes, nose, and cheeks induced by a combination of cold temperature and moisture. The lesions may ulcerate and may be associated with arthralgias. Histologically, lesions are characterized by unspecific vasculitic changes with deposits of complement and immunoglobulin and absent hyperkeratosis. The disease onsets in early childhood and tends to improve in late adulthood. Thus, the clinical and histological findings are consistent with either perniosis or Chilblain lupus, a rare cutaneous form of lupus erythematoses. Extensive investigation of 3 affected individuals of a multigenerational nonconsanguineous German kindred could exclude the presence of antinuclear antibodies, complement deficiency, cryoglobulinemia, cold agglutinins, infections, traumatic injury, keratolytic winter erythema, or lupus pernio.

Whole-genome linkage analysis was carried out on 25 family members including 16 affected individuals using the Affymetrix GeneChip Human Mapping 10K Array version Xba131. PedChek was used to detect Mendelian errors. Parametric linkage analysis was carried out with SimWalk 2 using a stepwise analysis of non overlapping marker sets covering 25-100 SNPs at a time. Assuming a fully penetrant autosomal-dominant trait the disease gene was localized within a 15 cM interval on chromosome 3p21-3p14 with a maximum location score of 4.7 for SimWalk 2 analysis.

Identification of the gene responsible for familial perniosis may shed light onto the pathogenesis of common forms of collagen vascular disease such as vasculitis or lupus erythematoses.

P0277. 13p and Yq homology have anything to do with male infertility status: A novel familial inheritance of 13p deletion**L. Rao¹, A. Babu¹, K. Murthy¹, M. Deenadayal², L. Singh¹;**¹Centre for Cellular and Molecular Biology, Hyderabad, India, ²Infertility Institute and Research Centre, Hyderabad, India.

Answers to deviations regarding the decline in fertility rate, and altered fecundability have gradually focused on individual genetic make up. The natural transmission of microdeletion syndromes is occasionally reported. We present an interesting finding in a family with four males and two females with inheritance of p arm deletion of chromosome 13 for the past four generations. This defect is predominant in males of the family, with oligoasthenoteratozoospermia and low intellectual abilities, whereas females with the defect are normal. The deletion was confirmed with G banding and fluorescence in situ hybridization.

This reveals genes on p arm of chromosome 13 have association with mental status and fertility aspects and is essential to define pathogenetic significance of chromosome 13p deletion. Molecular investigations showed normal Y chromosome. Studies are in progress to narrow down the region of chromosome deletion breakpoint and map the candidate gene(s) for the cause in this region. Thus allows understanding the possible mechanisms by which deletion might affect meiosis in spermatogenesis and lead to infertility.

Hypothetically, altered fertility rate observed among males in the four generations follows position-effect variegation (PEV) phenomenon. Sequence blast analysis of breakpoints in deletion region of chromosome 13p in affected individuals showed 85% homology with Yq region. Molecular characterization is currently underway to annotate underlying sequences with Yq region. This would further find novel relationship between autosomal aberrations and testicular dysgenesis or spermatogenesis arrest and map corresponding regions on each autosome in regard to recorded aberrations accompanying these disturbances.

P0278. Clinical presentation of mitochondrial encephalomyopathies**J. Pilch¹, E. Marszał¹, M. Kajor², E. Jamroz¹;**¹Department of Child Neurology, Medical University of Silesia, Katowice, Poland, ²Department of Anatomopathology, Medical University of Silesia, Katowice, Poland.

Mitochondrial diseases are a very heterogeneous group of congenital respiratory chain defects. Their diagnostics is difficult because of the diversity of clinical presentations. The authors report four children with mitochondrial diseases based on clinical picture and muscle pathomorphology. In the youngest patient with miopathic presentation first symptoms appeared at the age of 7 months. The next two boys presented with rapid fatigue associated with physical exercise and recurrent vomiting were observed at the age of 9 and 12 years. The mother of one of them is suffering from MELAS. In the last boy external progressive ophthalmoplegia was observed since the age of 9 years. All patients had hyperlactatemia. MRI of the head revealed abnormal disturbed myelination in three cases. Only in two patients myopathic pattern in EMG was noted. Based on the muscle tissue pathomorphology the ragged-red fibers were not found only in the youngest patient, but in that case the diagnosis was confirmed by the presence of structurally abnormal mitochondria. At all of them COX activity was negative and the respiratory chain enzyme activity was decreased. Nowadays, the molecular analyses of mtDNA are in progress.

P0279. Cerebral, cerebellar and colobomatous anomalies in three related males: sex-linked inheritance in a newly recognized syndrome with features overlapping with Joubert syndrome**H. Y. Kroes¹, R. A. J. Nieuvelstein², P. G. Barth³, P. G. J. Nikkels⁴, C. Bergmann⁵, R. H. J. M. Gooskens⁶, G. Visser⁷, J. K. Ploos van Amstel¹, F. A. Beemer¹;**¹Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands, ²Department of Radiology, University Medical Center, Utrecht, The Netherlands, ³Dept. of Pediatric Neurology, Emma Childrens Hospital/University Medical Center, Amsterdam, The Netherlands, ⁴Department of Pathology, University Medical Center, Utrecht, The Netherlands, ⁵Dept. of Human Genetics, Aachen University of Technology, Aachen, Germany, ⁶Department of Child Neurology, University Medical Center, Utrecht, The Netherlands, ⁷Department of Metabolic diseases, University Medical Center, Utrecht, The Netherlands.

We present a so far unrecognized X-linked mental retardation syndrome with features overlapping with Joubert syndrome.

Two brothers showed hypotonia, mental retardation, retinal colobomas and a breathing pattern compatible with Joubert syndrome. Neuroimaging revealed cerebellar vermis hypoplasia and ventriculomegaly. A tentative diagnosis of Joubert syndrome was made, and autosomal recessive inheritance considered most likely.

In a subsequent pregnancy that occurred after artificial donor insemination, ultrasound in the 22nd week revealed a Dandy-Walker malformation and hydrocephaly. At autopsy at 34 weeks of gestation, the male infant showed cerebellar vermis aplasia and abnormalities of the brainstem and cerebral cortex. He was considered to have the same disorder as his two half-brothers. This renders the pedigree highly suggestive of X-linked inheritance.

The clinical symptoms of this syndrome resemble Joubert syndrome. However, the absence of the molar tooth sign, the presence of supratentorial abnormalities, and the X-linked inheritance do not support Joubert syndrome. We propose the name X-linked cerebral-cerebellar-coloboma syndrome to distinguish the two disorders. Molecular analysis of several genes for X-linked syndromal mental retardation and Joubert syndrome is currently being performed. Differentiation of the two disorders is especially important in genetic counselling, where artificial donor insemination may be considered as a means of reducing the recurrence risk, or when female relatives of the patient are concerned.

P0280. Oculoauriculovertebral dysplasia with rare associations

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A case report of three year old female child whose parents are of non consanguineous origin, who are not consistently exposed to any of the potent mutagens during pregnancy and so the role of any of the mutagens on the phenotype is not clear and the karyotype analysis of the proband appears to be normal (46,XX) with contracted gall bladder, doubtful chloeseatoma, fusion of fourth and fifth cervical vertebrae along with various defects, mentioned in oculoauriculovertebral dysplasia or goldenhar syndrome which includes hemi vertebrate, facial palsy, limbal dermoid cyst, sensory neural hearing loss, microtia and middle ear anomalies. The impact of pathogenesis of oculoauriculovertebral spectrum might be the genes involved in notch signaling pathway, which plays an important role during somitogenesis are reviewed.

P0281. Trisomy 8p syndrome: A case report and review of clinical features

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Duplication of the short arm of chromosome 8, *de novo* or resulting from parental translocation, associated with craniofacial defects (high forehead, frontal or parietal bossing, carp mouth, full cheeks, and round face), brevicollis with redundant skin folds, mental retardation, absence of the carpus callosum, multiple minor skeletal abnormalities, and other abnormalities.

Here, a one month old male baby is described with duplication of 8p as the result of a maternal balanced reciprocal translocation. His karyotype was ascertained as

46,XY,der(13)t(8;13)(p11.2;p12). The father had normal karyotype. The mother had an apparently balanced translocation involving chromosome 8 and 13 (46,XX, t(8;13)(p11.2;p12).

Our case had macrocephaly with subtle changes in facial appearance. Pethosis of the left eye and micrognathia were also detected. The case had probably cortical blindness. The patient did not show any response to voice. The right parietal bone was prominent but not the left one. Right and left coronary artery in their origin and Aorta were dilated as detected by echocardiography. Brain CT scan showed agenesis of carpus callosum.

This case represents typical features of duplication 8p syndrome reported in the published cases. It should be noted that the phenotypic expression is related to the length of the duplicated segment. Furthermore, phenotype of dup8p resulting from a translocation is much more variable, probably because of different breakpoints. These findings, together with those reported cases with dup (8), define a syndrome that emphasizes the importance of genes on the 8p region for development of brain and heart especially.

P0282. Detection of chromosome 18 centromere instability and aneuploidy in Alzheimer patients by using Fluorescent in situ hybridization

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Premature centromere division (PCD) is an uncommon cytogenetic abnormality characterized with chromatides distinctively separated before anaphase movement. PCD or out-of-phase centromere division is found in ageing cells, Alzheimer disease (AD), various chromosome instability syndromes and cancers. It is also been proposed to be an important mechanism of aneuploidy (i.e. chromosomes 21 and X in AD patients). It is known that chromosome 18 separates first in metaphase - anaphase transition of the cell cycle. FISH analysis revealed that the aneuploidy in AD cells is not appearing only at chromosome 21, but at least chromosome 18 may exhibit trisomy as well.

The aim of this study was to estimate occurrence of PCD of chromosome 18. Peripheral blood lymphocytes were analysed in 6 AD patients (age 69.8±7.2) and 6 control subjects (age 69.8±7.5). FISH analysis for the alpha-centromeric probe of chromosome 18 on interphase nuclei has revealed that chromosome 18 expresses PCD in 2.59% in control group and 5.18% in AD patients, which is statistically significant ($p<0.05$) increase. Using FISH on interphase nuclei, our results show that PCD can occur earlier than metaphase of mitosis, i.e., in interphase of the cell cycle, immediately after replication. Our results showed a significant increase in hyperploidy (7.18% and 4.23%) and hypoploidy in Alzheimer patients compared to control subjects (8.21% and 5.08%), suggest that out-of-phase centromere separation can be seen as a manifestation of chromosome instability which may lead to aneuploidy.

P0283. Cytogenetic and FISH analysis in female patients with primary amenorrhea

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Sex determination and development in humans is a very complex process.

Many genetic or chromosomal events can alter the process and result in sex reversal.

XY females can become from an abnormal interchange between the X and Y chromosomes or from a mutation in SRY gene.

Cytogenetic analysis was carried out on 5 cases with primary amenorrhea, culturing peripheral blood lymphocytes and using GTG banding technique. Their age ranged from 17-37y. Chromosomal sexual assignment was established as 46,XY in 1 phenotypically female. One pt. had 46,XX / 47,XXY, while the rest had a female karyotype 46,XX.

All the pts received hormone-replacement therapy and therapy for induced osteopenia as well.

Using FISH technique, we used the LSI SRY(Yp11.3) s.o. and CEP X s.g./CEP Y (alpha satellite) s.o. probes (VYSIS).

The results confirmed the diagnosis of sex reversal in the younger pt. Four pts with CEP X / CEP Y probe confirmed their female sex, but with probe LSI SRY, specific for SRY gene at Yp11.3 region, it was found that about 30% of the interphase nuclei and more than three metaphases had a positive signal.

We believe that in our pts there is material of Y-chromosome carrying the SRY gene and even more that this is responsible for their primary amenorrhea.

We support the recommendation that FISH analysis with SRY probe in females with primary amenorrhea should become part of the routine investigation.

P0284. Ring Chromosome 4 Proven by FISH Study in a Child With Cleft Lip and Palate, Iris Coloboma, Mid-Gut Malrotation, Hypopspadias and Corpus Callosum Hypoplasia

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We report a 16-month-old mental and motor-retarded male patient with ring chromosome 4 and multiple congenital anomalies such as unilateral cleft lip and palate, iris coloboma, microcephaly, hypospadias and double urethral orifices. Peripheral chromosome analysis of the patient showed 46, XY, (r4) *de novo*. Multicolor FISH study was

also performed and according to multicolor banding (MCB) a r(4)(::p16.3>q34.3~35.1::) was found in all metaphases. Subtelomere 4p was present but subtelomere 4q was absent. Cranial MRI showed hypoplastic corpus callosum; delayed myelination; cortical atrophy. Chromosomal analysis of both parents were normal. Amniocentesis for the second pregnancy revealed a normal 46,XY karyotype. Phenotypically normal baby was delivered. Phenotypic features of ring chromosome 4 cases change according to deleted part of the chromosome 4 although severe growth retardation, microcephaly, cleft lip and palate are consistent features. Midgut malrotation as seen in our case was only reported in a mosaic form of ring 4 associated with short bowel and dextrocardia. In conclusion, the first ring 4 Turkish patient stressed that ring 4 chromosome anomaly should be suspected in every case with severe mental and motor retardation, microcephaly, hypertelorism, cleft lip and palate, corpus callosum hypoplasia and duodenal or midgut malrotation.

P0285. Evaluation of DNA damage induced by spilled *Prestige* oil exposure in a group of volunteers by means of comet assay

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After *Prestige* accident, in November 2002, about 63000 tons of mixtures of fuel-oil and sea water reached Galician coast, and more than 200 beaches (150 Km of coast) were seriously affected. As a consequence, birds were contaminated with the oil. As many as 10000 died, and 2500 were taken to the two centres for bird recovery in Santa Cruz (Oleiros) and O Campiño (Pontevedra), where they were cleaned and cured.

In this work, peripheral leukocytes from a group of young biologists involved in the cleaning of contaminated alive birds and in making the autopsies of dead birds were analysed by means of comet assay, in order to determine the DNA damage associated with exposure to the oil. A group of non-exposed students were used as controls. Additionally, volatile organic compounds were analysed in the ambient air of the work rooms by means of a gas chromatography coupled to ion trap mass spectrometry technique.

Results obtained showed significantly higher DNA damage in the group of exposed individuals, with comet tail length increasing with time of exposure. Individuals from O Campiño showed higher DNA damage than those from Santa Cruz, probably associated to their higher mean exposure time. No effect of protective measures (gloves, masks, overalls) has been detected, due to the low number of exposed individuals that did not use them.

P0286. Cytogenetic evaluation in a group of individuals exposed to *Prestige* oil during autopsies and cleaning of contaminated birds

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In November 2002 the oil tanker *Prestige*, loaded with 77000 tons of crude oil, broke up in the Northwest coast of Spain, spilling more than 63000 tons into the sea water. This ecological disaster unleashed a great mobilisation of volunteers to carry out the labours of cleaning beaches, sea, contaminated birds, etc. The *Prestige* oil is classified as fuel oil No. 6 by the USEPA, and the IARC regards this grade of oil as possible human carcinogen (group 2B).

In order to determine if exposure to *Prestige* oil causes cytogenetic damage, micronucleus (MN) test has been performed in peripheral lymphocytes from a group of volunteers that cleaned alive oil-contaminated birds and made autopsies of dead birds, and results have been compared with a control group.

No significant difference has been obtained between MN frequencies in the control and exposed groups, even at the higher time of exposure. Moreover, no effect of gender, age or smoking habits has been observed. These results suggests that protective measures adopted during the handling of birds have been suitable, at least to avoid any cytogenetic damage.

P0287. Cryptic subtelomere chromosomal rearrangement screening in 19 patients with mental retardation / malformation syndromes

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Background: Mental retardation affects 2-3% of the general population, with an unknown cause in more than 50% of the cases. Cytogenetically undetected chromosomal imbalances have been indicated as an explanation. In recent years, due to the development of molecular cytogenetic techniques, it became possible to identify cryptic rearrangements involving the ends of chromosomes. Subtelomeric aberrations have been identified as a significant cause of mental retardation and/or malformation syndromes. **Material and methods:** Here we report a subtelomere fluorescence in-situ hybridization (FISH) study of well-selected group of 19 unrelated children with mental retardation (MR), dysmorphic features, and a normal karyotype. The preliminary ascertainment checklist comprises evaluation of MR or developmental delay, dysmorphism, growth defect, and abnormal pedigree. Recognized dysmorphic syndromes have been excluded from the study. **Results and discussion:** Our study has found two *de novo* subtelomeric rearrangements, deletions of chromosome 4p tel and chromosome 13q tel, and one normal polymorphic variant of chromosome region 2q tel, a maternally transmitted deletion of 2q37. Clinical findings of patient 1 covered a well-defined Wolf-Hirshhorn syndrome features although the del(4)(p16.3) is cytogenetically undetectable. Patient 2 showing del(13)(q34) demonstrated growth and mental retardation, microcephaly, sloping forehead, bird-like nose, small chin, large ears, long fingers with wide terminal phalanges, heart defect, ventral location of the anus. **Conclusion:** Our findings showed 10.5% incidence rate of subtelomere rearrangements, which is comparable with previous reports on subtelomere abnormalities. This study highlights the importance of searching for cryptic subtelomeric rearrangements in non-syndromic mentally retarded patients.

P0288. Phenotypic and cytogenetic spectrum of chromosome 9p trisomy

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In this study we investigated three cases with *de novo* short arm duplication of chromosome 9.

The aim of this study was to identify the genotype / phenotype correlations and to use molecular cytogenetics to identify the exact duplicated part. Using GTG, DAPI stain, Whole chromosome paint, centromere, telomere and 9p21 specific locus probes demonstrated that in case one trisomy was due to translocation between chromosome 9 and 13 and duplication of 9p giving i(9p), the second case due to free 9p trisomy and the 3rd case due to duplication of whole short arm and part of the long arm (partial 9 trisomy). This study suggests the presence of hot points for chromosome 9 breakage at proximal 9q. Although both patients 1 and 2 had the same trisomy involving 9p, Case one with i(9p) exhibits the classical clinical manifestations of 9p trisomy such as mental retardation, ear anomalies, hypertelorism, bulbous nose, down-turned corners of the mouth, and hand/feet anomalies, while case 2 had overlapping features with Coffin-Siris syndrome. The significance of such observation may point to possible gene location to Coffin-Siris syndrome on 9p. Case 3 had additional manifestations more than those typical of trisomy 9p. This could be due to duplication of 9q21 region. Each case had unique clinical and cytogenetic manifestations, this may be due to different gene involvement according to the molecular site of the breakage point.

P0289. Small supernumerary marker chromosomes characterized by multicolor FISH techniques: database of Belarusian Registry of Chromosomal Abnormalities

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Marker chromosome is structurally abnormal chromosome in which no part can be identified (ISCN, 1995). Small supernumerary marker chromosomes (sSMC) differ depending on chromosomal origin, euchromatic DNA-content, degree of mosaicism, possible of

uniparental disomy presence. sSMC occur in about 0.045% of the human population (72/161536 newborn infants) (Liehr, 2004 http://mti.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC).

The characterization of sSMC is the important task of prenatal and postnatal cytogenetic diagnostics and genetic counseling, therefore a variety of special FISH approaches have been developed for that.

Totally 45 cases with SMC in constitutional karyotype were registered in Belarussian Registry of Chromosomal Abnormalities among the individuals who were cytogenetically examined in Republic Genetic Service during 1983-2004 years.

Comprehensive molecular cytogenetic methods were used for diagnostics of all available cases: M-FISH, cenM-FISH, microdissection of sSMC with subsequent reverse painting, acro-cenM-FISH, centromere-near BAC probe using, etc.

We present data of origin, structure and mosaicism status of 20 prenatal/postnatal sSMC cases diagnosed, describe the significant and rare clinical findings, and discourse the productivity of approaches used.

Karyotype	Phenotype
47,XX,+inv dup(15)	Normal
47,XY,+inv dup(15)	Normal
47,XY+inv dup(15)	Normal
47,XX,+inv dup(15)/47,XX,+r(15)/46,XX	Normal
47,XX,+inv dup(15)*	Normal
47,XY,+inv dup(15) mat*	CA
47,XX,+inv dup(15)	CA
47,XY,+inv dup(15)	CA
48,XXX,+inv dup(9p)/47,XXX	CA
47,XX,+inv dup(12p) (neocentromeric)	CA
47,XX,+inv dup(12p)/46,XX	CA
47,XY,+inv dup(18p)	CA
47,XX,+inv dup(22)	CA
48,XY,+der(19),+der(19)	CA
47,XY,+der(21)	Normal
47,XY,+der(14)t(11;14)(p15;q11.2)	CA
47,XX,+der(21)t(11;21)(q21;q22) mat	CA
47,XX,+der(22)t(11;22)(q23;q11) pat	CA
47,XX,+der(22)t(11;22)(q23;q11)	CA
47,XY,+der(22)t(11;22)(q23;q11) mat	CA

*- mother and son

CA- congenital abnormalities

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P0290. The different heteromorphic patterns in chromosome 9 pericentric region - towards a better understanding

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Among the non-acrocentric human chromosomes, chromosome 9 presents with the highest degree of morphological variations. Variants, also called heteromorphisms, like 9qh+, 9qh- or inv(9)(p11q13) are common findings in routine cytogenetics. In a previous study we characterized in summary thirteen molecularcytogenetically different heteromorphic pattern of the pericentric region of chromosome 9 (Starke et al., 2002; Eur J Hum Genet 10:790-800). In that study we used a set of three fluorescence in situ hybridization (FISH) probes: a chromosome 9 specific alpha satellite probe, a chromosome 9 specific classical satellite III DNA probe and a microdissection probe specific for 9p12/9q13-21.1. This probe set was enlarged in the meantime by so-called subcentromeric BAC-probes located in 9p12 and 9q13, respectively (probes are specified in Starke et al., 2003; Hum Genet 114:51-67). Thus, we were able to characterize 3 additional, previously unreported heteromorphic pattern of chromosome 9 and to describe variants like '9ph+' (acc. to Starke et al., 2002) in more detail. The biological and/or clinical significance of chromosome 9

heteromorphisms is still unclear. Connection with reproductive failure, mentally retardation, schizophrenia, the Walker-Warburg syndrome, the oculo-auriculo-vertebral (Goldenhar) spectrum and even with cancer predisposition were suggested throughout the literature. The now available possibility to distinguish by FISH between the different heterochromatic patterns hidden behind the cytogenetic finding of a 'heterochromatic variant of chromosome 9' will lead to a clearer genotyp-phenotype correlation of chromosome 9 heteromorphisms in future. Supported by the Dr. Robert Pfleger-Stiftung.

P0291. Three families with a chromosome 5 inversion

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We present three familiar occurrences of the chromosome 5 inversions. Routine prenatal cytogenetic analysis by G-banding was indicated by positive screening results in one case and advanced maternal age in two other cases. There were no spontaneous abortions registered in any of these families, only a vanishing twin syndrome was identified in one pregnancy.

Paracentric inversions: inv(5)(q23.2q33.3) and inv(5)(q22q35.2) in two families and a pericentric inversion: inv(5)(p12q13.2) in the third family were found. Breakpoints were verified by multicolor banding, supported by grant GACR 301/04/0407. Father was a carrier of the inversion in all of the three families.

We also try to investigate karyotypes of other family members. The results of the investigations can be effectively used in prenatal cytogenetic analysis at prospective pregnancies in other consanguineous relatives.

P0292. Comparing different molecular cytogenetics methods for detecting subtelomeric rearrangements

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The subtelomeric regions are interesting from a genomic perspective, as they are gene rich and often involved in chromosomal rearrangements. Most telomeres stain light with G-banding, and small rearrangements are therefore difficult to detect. Due to the development of molecular cytogenetic techniques, it is possible to identify cryptic rearrangements involving the ends of chromosomes. Screening method generally used for detection of subtelomeric rearrangements is multiprobe telomere fluorescent in situ hybridization (T-FISH). With T-FISH and other two methods, multiplex ligation-dependent probe amplification (MLPA) and comparative genomic hybridization (CGH) we analyzed subtelomeric regions of five patients, where the subtelomeric aberrations with T-FISH were found. Among them we found two de novo subtelomeric deletion: del(X)(ptel) and del(9)(ptel) and three unbalanced subtelomeric rearrangements (two among them were consequences of familial translocations): t(10;13)(qtel;qtel)-de novo; t(8;21)(qtel;qtel); rec(X)(qtel;qtel). MLPA confirmed all five subtelomeric rearrangements. With CGH we screened the whole genome of patients to find possible deletion or amplification. This technique confirmed previous founded subtelomeric anomalies which were bigger than 8 Mb.

Our study was used to determine the feasibility of these three methods for clinical testing on patients with mental retardation and/or developmental disabilities. We concluded that T-FISH and MLPA are both very useful and interchangeable methods with some exceptions. The choice of either method can be influenced by the technical conditions of the laboratory.

P0293. The first experience of bone marrow karyotyping in the private laboratory of Lithuania

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The culturing of bone marrow and karyotyping is a very popular and helpful method in diagnostic of hematological disorders. In Lithuania it was used in only a small area - for detecting of Philadelphia chromosome. The "Biomedical research center" is a private laboratory in Lithuania, first started in last year to perform a full karyotyping on bone marrow. In period from 2004 Jun to 2004 12 was performed 36 karyotypes from

bone marrow of patient with different age and diagnosis. The material was send from largest hematological clinics of Lithuania. The bone marrow was cultured according to standard methodic (direct and overnight culture; in some cases a more long culturing depending on preliminare diagnosis). In about 2/3 off all cases an abnormal karyotype was detected.

P0294. Detection of numeric chromosomal aberrations using multiplex ligation-dependent probe amplification

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Numeric chromosomal aberrations are usually identified with cytogenetic analysis. A variety of other methods can substitute karyotyping to detect aneuploidy but all have downsides; some are time-consuming, labor-intensive, some require high quality DNA, etc. Recently, a method, multiplex ligation-dependent amplification (MLPA), was described which can be used to detect the correct number of all chromosomes in the small amount (50ng) of genomic DNA in a single reaction by measuring the gene dosage differences. The method relies on amplification and quantitation of probes added to the test sample. In this study, MLPA was compared with cytogenetic analysis of cultured fetal tissues.

Genomic DNA was extracted from 24 samples from spontaneously terminated pregnancies routinely sent to cytogenetic analysis. All samples were cultured and when possible karyotyped. The MLPA analysis was performed with the subtelomeric kit from MRC-Holland which includes one specific probe per chromosomal arm in a single reaction.

The cytogenetic analysis confirmed the chromosome number established with the MLPA analysis in 17 samples. Several common numeric chromosomal aberrations were detected. Lack of cell growth precluded karyotyping in 7 samples where only MLPA results were obtained.

The MLPA analysis can be successfully used to detect the correct number of chromosomes and is therefore suitable as a diagnostic method for numeric chromosomal aberrations. Also it can provide partial information on karyotype of a tested sample when cytogenetic analysis is not possible because of lack of viable cells or when only a small amount of template DNA is available.

P0295. deletions and duplications of distal 15q: low/tall stature and speech abnormalities

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We defined at BAC level through array-CGH six cases with different distal 15q deletions/duplications.

Age ranges 18-38 years but a 8-years-old girl. Cases 1-5 have a 2.2-4.8 Mb deletion secondary to a ring. All have mild mental retardation, speech delay, articulation difficulties, obesity, stature <3rd centile. Case 6 is tetrasomic for the distal 4.5 Mb due to an invdup analphoid marker. She has a borderline intelligence but suffers from severe dysarthria (inability to pronounce some consonants and difficult-to-understand speech), stature >97th centile.

Both low and tall stature are reported in distal 15q imbalances and attributed to dosage effect of the IGF1R gene. Missense and non-sense IGF1R mutations result in intrauterine and postnatal growth retardation. In cases 1, 2, 5 IGF1R was included in the deletion whereas in cases 3, 4 the deletion breakpoint was at 500 kb from the IGF1R 3' where a regulator of IGF1R expression might be located thus explaining the low stature of cases 3, 4. Case 6 marker contains two dosages of IGF1R. These cases suggest that IGF1R is dosage sensitive and that the final stature is strongly influenced by haploinsufficiency or tetrasomy for this gene. Most distal trisomy 15q cases are reported with overgrowth further supporting the hypothesis. All the subjects suffer from speech-articulation disorders. This was especially evident in case 6 because of

her almost normal intelligence. Although an assessment of their verbal performance is in progress, we hypothesize that a dosage-sensitive gene, responsible for speech disorders, is located in the very distal 15q.

P0296. The evaluation of genotoxic potential of Tc-99m O₄⁻ in lymphocyte culture of patients with thyroid disease

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The genotoxicity study of Technetium-99m pertechnetate (Tc-99m O₄⁻) was carried out on human lymphocyte chromosomes using chromosomal aberrations (CA) and micronucleus (MN). Exposure to ionizing radiation may result in DNA damage giving rise to chromosome alterations and complex chromosome rearrangements. Cytogenetic tests play an important role in the detection of biological effects of low doses of radiation in patients exposed to ionizing radiation. CA and MN were measured before, 1 hour and one month after Tc-99m O₄⁻ administration. A statistically significant increase was observed in the SCE and MN frequencies 1 hour after Tc-99m O₄⁻ administration could be described. It has been obtained any significant difference in CA and MN frequencies one month after Tc-99m O₄⁻ administration. The results of the study suggest that Tc-99m O₄⁻ might moderate genotoxic potential.

P0297. Balanced chromosomal rearrangements in couples with reproductive failure

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Balanced chromosomal rearrangements are known to be a risk factor for both recurrent pregnancy loss and infertility, not only by predisposing to meiotic malsegregation of chromosomes and imbalanced gamete formation, but the abnormal meiotic configurations arisen by homologous pairing of translocation chromosomes may also disrupt gametogenesis, mostly in males.

We carried out cytogenetic analysis in 130 couples referred for recurrent spontaneous abortions and 40 infertile couples. Chromosome examinations were performed on cultured peripheral blood lymphocytes, using GTG-banding. In the group with repeated miscarriages we detected a simple translocation t(4;12) in a woman, two reciprocal translocations: t(2;11) in a woman and t(4;16) in a man; an insertional translocation involving chromosome 3 in a female. We identified three carriers of a robertsonian translocation (two women and a man), three pericentric inversions of chromosome nine (two in the female and one in the male partner, respectively) and a paracentric inversion of chromosome 15 in a woman. In the group referred for infertility we found four carriers of a balanced chromosomal rearrangement: a t(4;X), a t(7;14) in mosaicism and a pericentric inversion of chromosome 10 in the female partners and a pericentric inversion of chromosome 9 in a male.

In our study the overall frequency of balanced chromosomal rearrangements was 7.6 % among the couples with recurrent abortions, affecting the female partner in 5.3 % of the cases. The high proportion of female carriers in the infertile group suggests that balanced structural anomalies would frequently lead to impairment of gametogenesis in females as well.

P0298. A Greig Syndrome patient with 46,XY,t(7;10)(p13;q11.2) karyotype

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A nine month-old male patient, the second child of a non-consanguineous couple was referred to our laboratory for cytogenetic

analysis because of polysyndactyly of hands and feet and minor dysmorphic facial findings. Chromosome analysis results revealed that the patient had an apparently balanced translocation involving the short arm of chromosome 7 and the long arm of chromosome 10. The karyotype was reported as 46,XY,t(7;10)(p13;q11.2). The parents' karyotypes were found to be normal. As the 7p13 region contains the GLI3 gene, the patient was reevaluated for GLI3 gene associated phenotypes. Differential diagnosis was made relying on the absence of corpus callosum agenesis and normal neuromotor development.

P0299. Detection of t(13q;14q) in a man with primary infertility

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Robertsonian translocations are one of the balanced structural chromosome abnormalities in infertile men. They present usually without an abnormal phenotype for the carrier. In men, they may result in abnormal sperm production. Both morphological and numerical abnormalities in sperm have been reported in t(13q;14q) carriers. A 34 year old men with primary infertility due to azoospermia was referred to our department for cytogenetic and Y chromosome microdeletion analysis. We detected the karyotype as 46, XY, t(13;14)(q10;q10). Y chromosome microdeletion was not detected in the screened regions. Pathological examination of testis biopsy specimens revealed incomplete spermatogenesis. t(13q;14q) has been reported to be together with abnormal spermatogenesis. Sperm production is affected by chromosomal pairing in meiosis and the sex vesicle affects the sperm morphology. The patient was informed about the condition during genetic counselling.

P0300. Molecular Cytogenetic Analysis of inv dup(15)Chromosomes Observed in Two Patients with Different Clinical Manifestations

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Supernumerary marker chromosomes (SMCs) of chromosome 15, designated "SMC(15)s," are the most common SMC in humans, accounting for as much as 50% of all those observed. In this study we report two cases with de novo large SMC(15) (inv dup 15 including PWACR), both cases were referred for delayed physical and mental milestones but had different phenotypic manifestations. Our aim is to clarify the genotype-phenotype correlations. Conventional cytogenetic analysis of their peripheral blood lymphocytes revealed the presence of SMC in all cells (47,XY,+mar). FISH analysis using whole chromosome paint 15 showed that SMCs were derived from chromosome 15. Using centromeric 15 and SNRPN specific locus probes in 15q11-13 showed that the marker in both of them had two copies of chromosome 15 centromere and two copies of SNRPN locus. So the marker could be described as idic(15)(pter,q13). Both cases were males of non consanguineous marriage, clinically they exhibited microcephaly, profound mental retardation, convulsions, and behavioural changes, and they showed no clinical manifestations of Prader-Willi or Angelman syndromes. Although both cases had similar markers, one case showed no dysmorphic features, while the other had marked craniofacial anomalies and neurological manifestations. This clinical discrepancy might be due to different breakpoints involved.

P0301. Repeated spontaneous abortions due to balanced reciprocal translocation, 46,XX ,t(6;15)(q23;qter) - Pathogenesis of duplication/deletion of chromosome 6q : A case report and brief review

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Pregnancy loss, particularly early miscarriage, is a common problem that affects many women and their partners. Most spontaneous miscarriages are caused by an abnormal karyotype of the embryo. At least 50% of all first-trimester SABs are cytogenetically abnormal. Many couples are anxious to understand why miscarriage has occurred and are hesitant to pursue another pregnancy until they understand more about the cause of their loss. Cytogenetic evaluation of the couple often

helps to alleviate concerns about recurrence risk in future pregnancies. In many cases, monitoring of future pregnancies can lead to the birth of a healthy baby. Reciprocal translocation in one of the partners is one of the most frequently observed structural chromosomal abnormalities as one of the major causes for repeated fetal loss.

A young healthy couple (Syrian) with history of 3 first trimester abortions was investigated. Karyotype of the husband showed normal 46, XY. A balanced reciprocal translocation, 46, XX, (6;15) (q23;qter) was observed in the spouse. Family history revealed several repeated abortions (7 times) in her two elder sisters and two abortions in her mother, and history of a living male child among close relatives, with mental retardation suggesting familial translocation. Translocations involving chromosome 6q and 15 are very rare. Prenatal diagnosis and PGD options were explained to the couple. The pathogenesis of trisomy 6q and monosomy 6q resulting meiotic segregation of parental balanced translocation will be presented.

P0302. Partial trisomy 11q with a rare paternal inheritance

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An eight year-old male was referred to our department with the complaints of mental retardation, inability to walk and speak. He was the second child of the non-consanguineous parents and having a 16 years old healthy brother. His examination revealed low frontal hairline, anteverted prominent ears, arched eyebrows, epicanthus, thick alae nasi, beaked nose, prominent nasal bridge, cryptorchidism, narrow feet and wide spaced 1st - 2nd toes, pes planus and equinovalgus deformity. He had severe mental and motor retardation and hardly walk only when supported in ante-flex position, and he has stereotypic upper extremity movements. The cytogenetic evaluation of the case and the parents revealed unusual rearrangements which were confirmed with FISH. His karyotype was 46,XY,-3,+der(3) dir ins(3)(3;11)(p13;q23-q24). The karyotype of the mother was normal and the father has 46,XY,ins(3)(3;11)(p13;q23-q24) chromosomal constitution. As he was a rare case of the medical literature with paternally inherited insertional translocation, his phenotypic features and cytogenetic findings are presented.

P0303. Mining novel oncogene rearrangements in leukemia-lymphoma cell lines

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Many oncogenes have been ascertained by molecular analysis of chromosome translocations recurring in patients with specific neoplasms, mainly leukemia-lymphoma (L/L) but only rarely in derived cell lines. The DSMZ has collected well characterized cell lines established from L/L patients, subjecting these to cytogenetic breakpoint analysis using tile-path clones, supported by gene-expression analysis. In addition to "standard" translocations, such as t(9;22)(q34;q11) in chronic myeloid leukaemia, several novel rearrangements juxtaposing known with unknown or rare oncogenes have been found. These include: t(6;7)(q25;q36) with HLXB9/MYB in acute myeloid leukaemia; t(14;17)(q21;q32) with HOXB5/IGH, t(3;7)(q27;q32) with FRA7H/BCL6, inv(3)(q25q27) with MBNL1/BCL6, t(8;9)(q24;p15) with PAX5/MYC, and t(9;14) with PAX5/VRK1 in B-cell lymphoma; and t(5;14)(q35;q32) with BCL11B/NKX2-5 in T-cell acute lymphoblastic leukaemia. Apart from t(8;9), none of these translocations has been described previously. Interestingly, most breakpoints coincide with highly conserved, non-coding DNA sequences associated with transcriptional regulators which may be disrupted by the associated translocations. Several independent lines of evidence suggest that the same rearrangements occur in vivo. First, all changes are either cryptic or documented at early passage; second, all novel oncogenes have close homologs involved in other tumor types; and finally, the first of these rearrangements discovered - t(5;14), has now been detected clinically. Thus, in addition to illuminating novel aspects of tumor biology, molecular cytogenetic analysis of L/L cell lines may inform diagnostic analysis.

P0304. Differential replication of the Y chromosome: study of different regions by FISH.

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Recent studies have shown that replication timing reflects gene expression. A gene replicated early in S phase will be expressed in the cell, whereas a gene replicating late will not be expressed. Also, actively expressed genes seem to have a loose chromatin structure as opposed to inactive genes which show a more condensed chromatin organization. To date, few loci have been studied according to these two aspects. We hypothesize that a correlation between replication timing and chromatin condensation exists. The first objective of this study is to determine the replication timing of different regions of the Y chromosome. To reach this goal, we are using a FISH replication assay and bacterial artificial chromosome (BAC) probes covering the Y chromosome. This variant FISH technique can determine the replication time of a DNA sequence in interphase nuclei of unsynchronized normal lymphocytes. We found a majority of late replicated BAC sequences and only a small proportion of BACs showing early replication timing. It seems that late replicated sequences contain either only one ubiquitously expressed gene, genes exclusively expressed in testis, or no gene. Instead, early replicated BACs include more than one ubiquitously expressed gene, and the presence of two adjacent ubiquitously expressed genes seems to define an early replication time zone. With this project, we want to establish the existence of replication domains and correlate these data with chromatin structure and DNA rearrangements. *Supported by RMGA-FRSQ and the Fondation de l'Hôpital Sainte-Justine.*

P0305. Value of karyotype in the exploration of couples with recurrent spontaneous abortions

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Recurrent spontaneous abortion (RSA) is defined as the occurrence of two or more consecutive foetal losses. It represent 0,94 % of all pregnancies and 9,78 % of spontaneous abortions. At the present time, cytogenetic analysis has been gained an important place in the etiological screening of RSA.

In this study, we report the cytogenetic findings of 39 couples with the history of first trimester repeated miscarriage (2 to 6). From May 2002 until December 2004, karyotype of 78 subjects has been performed in our Pasteur Institute's Cytogenetic Laboratory. The frequency of chromosomal abnormalities was found to be 7,6 %, i.e. 6/78 individuals, equally divided into autosomal structural rearrangements (reciprocal translocation, inversion) and sex chromosomal mosaicism (Turner's syndrome, Klinefelter's syndrome). No correlation between the distribution of the chromosome abnormalities and the number of abortions was observed.

In conclusion, we insist on the advantage of systematically performing a karyotype in the case of RSA, consisting on the detection of chromosomal abnormalities. Therefore, genetic counselling should be offered to these couples and investigations conducted on their extended families.

P0306. De novo ring chromosome 7 with LMMC

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Ring chromosome 7 is an unusual chromosome anomaly.

Here we describe a 5 year old patient with ring chromosome 7 who was referred for aplastic anemia. Subsequent cytogenetic techniques demonstrated a monosomy 7 with trisomy 8 in cultured bone marrow cells, while the lymphocyte culture showed 3 kinds of cells: a cell line with large ring chromosome replacing one of the normal chromosomes 7 resulting in partial monosomy in the majority of cells, 80% percent of

the analysed cells showed a ring chromosome composed of both the long and the short arms, r(7) (p22q36). In the remaining metaphases, we found a double ring chromosome 7, a monosomy 7, and two normal cells with 2 chromosomes 7. The girl had a developmental delay (growth retardation) and skin lesions.

Karyotype of the parents were normal confirming de novo origin of the ring chromosome formation.

To our knowledge, these uncommon cytogenetic abnormality have not been previously reported with LMMC.

P0307. Importance of cytogenetic investigation of 165 severely infertile men candidates for intracytoplasmic sperm injection (ICSI)

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Cytogenetic alterations represent a major cause of severe spermatogenic impairment leading to male infertility. The incidence of chromosomal abnormalities was estimated between 7 and 14%. The risk of transmission of chromosomal diseases by ICSI is therefore very high.

We report here the results of cytogenetic analysis of 165 infertile men with severe oligospermia, oligoastheno or oligoasthenotérapospermia and azoospermia referred to our laboratory of Cytogenetics of the Pasteur Institute of Tunis from April 2002 to December 2004. Chromosomal analysis was performed from peripheral blood lymphocytes cultures using RHG and fluorescent in situ hybridization (FISH) and karyotyping done using the Image Analyzer.

In 20 (12%) cases, chromosomal abnormalities was found. Whereas the 47,XXY chromosome complement was the commonest, the following abnormal karyotypes were also found: 2 XX men, a deletion of the long arm of the Y chromosome, chromosomal translocations found in 5 cases (Robertsonian translocations in 4 cases and a balanced reciprocal translocation in the last) and a chromosomal inversion.

All men with chromosomal abnormality were referred for genetic counseling.

These results show that cytogenetic screening and counselling are highly recommended in infertile males before referring to assisted reproductive techniques (ART) to avoid transmission, persistence, or even an increase of genetic defects in future generations.

P0308. Should chromosome breakage studies be performed in all patients referred for aplastic anemia?

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Aplastic anemia (AA) is referred to situations in which bone marrow fails to generate blood elements. This entity is composed of several different diseases and syndromes such as Fanconi anemia (FA).

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by congenital abnormalities, progressive bone marrow failure and cancer susceptibility. The lymphocytes and fibroblasts of FA patients show increased sensitivity to alkylating agents such as mitomycin C (MMC) and Diepoxybutane (DEB), generating increased chromosome breakage.

In this study we have investigated cytogenetically 43 patients referred for AA. Mitomycin C with 2 different concentrations (50, 80 ng/ml) and/or DEB have been applied to the lymphocytes of the patients and normal controls.

According to the criteria of MMC (and /or DEB) sensitivity only 7 cases were classified as FA patients.

All these cases showed the pattern of breakage characteristic for FA (>70% of aberrant cells).

The diagnosis of Fanconi anemia being of importance for genetic counseling and early therapeutic intervention in patients, we conclude that chromosomal breakage studies should be performed in aplastic anemia especially if the child also present skin pigmentation abnormalities, growth retardation, microcephaly or dysmorphism.

So, the diagnosis of FA can be made unequivocally by combining both the clinical data and the cytogenetic evaluation of chromosomal breakage induced by DEB or MMC.

P0309. Cryptic Chromosomal Abnormalities in Children with Mental Retardation Using Subtelomeric FISH

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Final diagnosis in mental retardation (MR) could be reached in approximately one third of all cases, and no diagnosis is achieved in the rest of the patients, which is followed by repeated laboratory tests and a long time exhausting both parents and the patients. Recent studies have established that chromosomal abnormalities comprise 30-40 % of moderate to severe MR; and nearly 30 % of mild MR. Subtelomeric rearrangements account for a significant proportion (2.2-23%) of cases with undiagnosed MR because of high concentration of genes in this region. The aim of this study is to screen a selected group of children with idiopathic MR for subtelomeric abnormalities using FISH.

A total of 30 children aged 3-16 years with idiopathic MR and normal karyotypes were included in this study. The children whose parents had consanguineous marriages were excluded from the study. All cases were evaluated using the scoring system published by de Vries et al. Terminal deletion of chromosome 9p in a case was detected by FISH (3.3 %). Higher resolution G-banding showed the same abnormality as well.

In conclusion the frequency of subtelomeric abnormalities in our study group was lower than the frequencies reported in other studies. Higher incidence of consanguineous marriages resulting higher frequencies of mutant alleles for autosomal recessive disorders may be the reason for this result. The scoring criterions were not found to be convenient to select the patients for subtelomeric FISH. High-resolution G-banded analysis in those cases and more effective selection criterions for FISH are suggested.

P0310. Ambiguous ISCN 1995 nomenclature causes problems with karyotypes - a CyDAS based analysis

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Thorough analysis of grammar, syntax and meaning of ISCN elements and their combinations was required during development of a software system for the analysis of ISCN data. Though ISCN 1995 allegedly was developed with considering computerized analysis, some inconsistencies and features exist which lead the user of ISCN to errors.

Designating bands involved in rearrangements is optional and bands are included in an extra pair of brackets separated from their chromosomes. Thus chromosomes and bands may be put into non-consistent positions, causing hardly detectable errors.

The use and positioning of multiplicators is purely chaotic, sometimes they precede the aberration, sometimes they follow the aberration with a multiplication sign inbetween, sometimes the aberration is to be shown twice or their duly use remains unclear.

The short nomenclature is often regarded as the ideal solution, but it is severely incomplete from a mathematical point of view. All its operators (the symbols describing aberrations) actually expect non-derivative chromosomes as input, and short nomenclature boldly fails as soon as an aberration spans a junction in a derivative chromosome. The long nomenclature still copes with most of such rearrangements and its use ought to be encouraged.

The description of an involvement of a centromeric fragment in a rearrangement is totally missing in the ISCN, e.g. duplication of pericentric regions or insertion of a centromeric fragment.

In summary, the project caused a new view on the nomenclature of cytogenetic findings which may lead to an improved ISCN.

P0311. Cytogenetics Results in 151 Iranian Patients with Mental Retardation

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Mental Retardation (MR) has heterogeneous aetiology, mostly with genetic causes. 7-8 percent of all MR patients and 50-60 percent of severe type have chromosome abnormalities. 151 MR patients were referred to Genetic Research Center for cytogenetics investigation. Standard Cytogenetics techniques using high resolution and GTG banding was carried out on all patients. The overall chromosome abnormality rate was 15 percent. The chromosome abnormality rate other than trisomy 21 was 6 percent. The abnormalities include a balanced reciprocal translocation between chromosomes 4 and 10, maternally inherited; an abnormal Yp; add(9)(p34); del(18)(p11); inv(1)(p33p35) and partial deletion of 10p. The significance of these chromosomal findings in mental retardation aetiology will be presented.

P0312. Chromosome instability in children with thyroid pathology born to irradiated parents due to Chernobyl accident

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We examined two groups of children with and without thyroid pathology born to parents irradiated by iodine owing to the Chernobyl accident who lived in the territory of Ukraine with high level of food contamination by Cs. Chromosome aberrations in peripheral blood lymphocytes were studied after 48 and 144 hours of culture by conventional preparation and G-banding. With short-term cultivation and conventional cytogenetics the means did not differ significantly between the groups. In both groups simple aberrations mainly of chromatid type were dominant. Mean-group frequencies of unstable exchanges (dicentrics and centric rings) corresponded to their age norm, but mean-group frequencies of stable chromosome aberrations (abnormal monocentrics) were significantly elevated above the spontaneous level. G-banding cytogenetics in short-term cultures increased the detection of chromosome aberrations (terminal and interstitial deletions and translocations) especially in children with thyroid pathology. The increased frequency of stable aberrations can be considered as a bioindicator of accumulated internal irradiation as well as a biomarker of transmissible chromosome instability. The latter have been confirmed in long-term cultures from children with chronic thyroiditis, in which significantly increased levels of both chromatid (single fragments) and chromosome (abnormal monocentrics) aberrations have been established. We assume that delayed cytogenetic effects expressed in children born to irradiated parents under short-term cultivation and G-banding cytogenetics as well as under long-term cultivation and conventional cytogenetics may be one of the factors which promote the development of thyroid pathology.

P0313. Case Report: A child with Rubinstein-Taybi phenotype and partial deletion of short arm of chromosome 10

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A seven year old boy with Rubinstein-Taybi features was referred to Genetics Research Center for Cytogenetics investigation. The clinical features included CHD, downslanting palpebral features, protruding tongue, broad thumbs and toes and moderate mental retardation. Standard Cytogenetics techniques using high resolution and GTG banding was carried out. The karyotype was abnormal showing a partial deletion of short arm of one chromosome 10 at p11.2-p13. Both parents had normal karyotypes. As about only 10 percent of Rubinstein-Taybi cases demonstrate deletion of 16p13.3, this chromosome anomaly could introduce a new candidate loci for this condition. To our knowledge, this is the first reported case.

P0314. Chromosomal rearrangements in Armenian registry of chromosomal abnormalities

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A total of 626 cases were investigated for suggested chromosomal abnormalities. We have studied a spectrum and clinical data of chromosomal rearrangements (ChR) detected by GTG-banding and FISH methods among couples with reproductive failures (204 individuals) and patients with malformations, mental and motor retardation, amenorrhea, hypogenitalism (422 children).

In the group with reproductive failure carriers with balanced chromosome rearrangements were found in 12 cases (6%). These chromosome aberrations include: 2 Robertsonian translocations, including t(13/14), t(14/21), 4 reciprocal translocations and a case of complex chromosomal rearrangement 46,XX,der(1;2)(p21;p23), inv(9)(p13;q13), 2 cases with inv(9)(p13;q13). Apart from the balanced aberrations, 2 cases with del(9)(p13;q13) without any phenotype abnormalities were found. The translocation 46,XY,t(4;9)(q26;p23)mat has been resulted in unbalanced translocation in the third generation with partial trisomy of the region 4q26-4qter.

Among children with birth defects karyotype abnormalities were found in 73 cases where 11 of them are presented with unbalanced ChR. Unbalance of autosomes was defined in 42 cases with Down syndrome of which 39 cases were represented with regular trisomy of 21, and 3 cases of the syndrome was due to translocation t(14/21)mat, combination with inv(9)(p13;q13)pat and full double aneuploidy 48,XY,+21,+mar. Using FISH analysis it has been shown that this marker was excluded to be 13/21 and Y chromosomes. A rare form of 49,XXXXY have been observed in unbalances of gonosomes. The other structural chromosomal aberrations include: 47,XY,+i(18p); 46,X,i(Xq); 46,XY,t(5;13)(q23;q32); 46,XX,t(9;11)(p24;q12); 46,XX,del(20q); 46,XY,inv(6)(p21.1;q21); 46,XY,inv(9)(p13;q13)pat.

Cytogenetic and clinical data will be presented and problems of genetic counseling for families will be discussed.

P0315. Atypical Down syndrome phenotype in a girl with translocation trisomy 21

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We described a 10 year- old girl with a de novo 21;21 translocation trisomy 21 and an atypical phenotype for Down syndrome. Clinical findings included microcephaly, small stature, coarse facies, epicanthus, hypertelorism, broad nasal bridge, low set ear, pectus excavatus, lumbar scoliosis, severe developmental delay, seizures, and hypertonia. Chromosome analysis from blood lymphocyte revealed 46,XX, t(21;21),+21. Fluorescence in situ hybridization (FISH) analysis confirmed the trisomy 21 translocation. Chromosome and FISH analyses were performed on skin fibroblasts. These studies revealed mosaicism for a translocation trisomy 21 cell line (53%) as well as a second cell line consisting of one normal chromosome 21 and a ring chromosome 21 derived from translocation 21q21q (33%) and a third line consisting of monosomy 21 (14%). We considered that our patient which was an atypical Down syndrome phenotype originated from chromosomal mosaicism of the skin and the other tissues that were not available for analysis.

P0316. The Lithuanian collection of 224 Turner syndrome patients.

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The collection was piled up during 30 years and contains 224 karyotypes. One half of the patients (116) had X monosomy 45,X. Chromosome mosaic 46,XX/45,X was established for 29 patients (13,0%). The commonest structural aberration was i(Xq), established for 42 patients (18,7%). For 16 patients it was the unique clone, for others different mosaic variations were found. Ring X chromosome was found for 19 patients (9,5%) and all of the patients had another cell clone, usually monosomic. Mosaic 46,XY/45,X was found for 8 patients (3,5%), in three of them another chromosome anomalies were established. The biggest established number of cell clones in mosaics was four: 45,X/46,X,r(X)/46,X,i(Xq)/47,X,i(Xq),i(Xq). Two monosomic patients were identical twins. The more often patients

for karyotype analysis were sent by endocrinologists (67.1%) and gynecologists (16.2%). The oldest patient was 52 years old, for six patients monosomy-X was suspected by neonatologist.

P0317. A rare variant translocation t(1;22;9)(p32;q11;q34) in a patient with chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is genetically characterized by the presence of the reciprocal translocation t(9;22)(q34;q11) in 95% of cases, resulting in a BCR/ABL gene fusion. About 5% of cases of CML show cytogenetic variants of this aberration.

In this study we report on a patient with CML with a rare complex rearrangement t(1;22;9)(p32;q11;q34) with BCR/ABL gene fusion on der(22). The male patient is 27 year old. His complete blood count showed a trombocytosis, a hemoglobin level of 97g/l, a white blood cell count of 342x109/l, myelocytes 102,6x109/l, bands count of 85,5x109/l, metamyelocytes count of 44,46x109/l, eosinophiles count of 6,84x109/l, lymphocytes 6,84 x109/l, basophiles count of 10,26 x109/l, polymorphs count of 68,4x109/l, blasts count of 17,1x109/l. Splenomegaly was also detected.

Cytogenetic studies were performed on 24h unstimulated cultures of bone marrow cells using standard cytogenetic protocol. Karyotypes were analyzed according to International System for Human Cytogenetic Nomenclature. Fluorescence In Situ Hybridization (FISH) was done according to standard protocol using probes WCP (Whole Chromosome Painting) 1, 9, 22 and M-BCR/ABL.

Conventional cytogenetic investigation detected derivative 22 and 1 chromosomes, while chromosome 9 was apparently normal. Application of WCP 1, 9, 22 permitted us to identify translocation between chromosome 1 and 22. After using M-BCR/ABL probe was found chimerical gene BCR/ABL on derivative 22 in 100% cells.

According to previous literature, despite their genetically complex nature, variant Ph chromosome rearrangements do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph chromosome.

P0318. Chromosomal investigation of a case initially diagnosed as Burn-McKeown syndrome

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Burn-McKeown Syndrome is a rare genetic disorder. Burn et al. (1992) reported five children who presented with bilateral choanal atresia and a spectrum of additional malformations including cardiac defects, deafness, defects of the external ear, eyes and eyelids, and a characteristic dysmorphic appearance. Our patient is a boy, aged 1 year 20 days, born after an uneventful term pregnancy. Positive findings in his physical exam are membranous choanal atresia, stenosis, hypotonia, chest deformity, short nose, open mouth, prominent forehead, mild low set ears, downward palpebral fissures, high and narrow palate, dental delay, low weight. He had growth and developmental retardation. The family pedigree of this child shows that his parents are distantly related with, no other similar family history.

In our lab we performed chromosomal analysis on standard PHA blood cultures. GTG banding was applied to air dried slides. The karyotype, 46XY,r(18) showed the presence of a ring chromosome. Interpretation of the banding pattern showed possible breakpoints in p14 and p23. Although autosomal recessive inheritance had been suggested, other studies (2003) raised the possibility of X-linked inheritance, because all reported patients, except for a female with a similar ring chromosome, have been male. The presence of the ring chromosome 18 in our case, and one of the initially reported cases suggests the possibility of a gene in the deleted region that could produce a phenotype similar to Burn-McKeown syndrome. Further analysis including FISH and CGH seems to be warranted and are being performed.

P0319. A retrospective review of cases with Pericentric inversion Y

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In the past 3 years, 31 cases of pericentric inversion of chromosome

Y have been identified in the males being referred for various reasons to our center. In an attempt to recognize or help clarify the significance of the inverted Y chromosome, these cases have been categorized according to available clinical data. All chromosomal studies are done on GTG banded metaphase spreads prepared following culture, harvest, spreading and trypsin banding. In all cases at least 5 spreads are analyzed, 10 spreads screened and 5 spreads scored. In the cases, where inversion Y was suspected, C-banding was done for confirmation.

4 cases were detected in amniotic fluid samples showing male fetuses with pericentric inversions of Y chromosomes. In all these 4 cases, the fathers were also studied and showed to have the same inversion Y. Four other cases were male aged 1 to 5 years with congenital anomalies, whose fathers were also examined and found to have the same chromosome Y.

Two cases had been referred for check up and without prior complaint.

The remaining 13 cases had been studied for history of abortions in their spouses and/or inviability of offspring. The above findings would appear to confirm that apart from a possible complication that the inverted chromosome Y has in crossing over with the pseudoautosomal region of the chromosome X, it is associated with no further specific clinical significance and occurs with similar frequencies within the various referral groups.

P0320. Down syndrome due to familial reciprocal translocation

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A two-year-old boy had typical Down syndrome clinical findings and developmental delay. The patient was born to a 24-year-old mother and a 25-year-old man, and he is the first child of the family. His karyotyping showed 47,XY,t(2;21)(q23;q11.2),+21. To confirm the translocation; fluorescent in situ hybridization (FISH) with a 21q22 specific probe was used and observed signals on both normal 21 chromosome and the der. We performed the chromosomal analysis of the parents. Maternal chromosomal analysis was normal but the father's chromosomal analysis revealed a balanced translocation, 46,XY, t(2;21)(q23;q11.2). Down syndrome had occurred as a result of an interchange trisomy following 3:1 malsegregation of the 2;21 balanced reciprocal translocation of the father. Down syndrome due to familial interchange reciprocal translocation is so rare, there is only 24 cases were published.

P0321. DiGeorge/Velo-cardio-facial syndrome detected by FISH

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We are reporting a case of DiGeorge/Velo-cardio-facial microdeletion contiguous syndrome detected by use of Cytocell DiGeorge/VCFS TUPLE1 probe. The proband is a 2 year old female, second child of unrelated couple with a 7 year old healthy brother. The only positive findings are nasal regurgitation and cleft soft palate, cleft upper lip and nasal speech.

PHA stimulated lymphocytes from peripheral blood were cultured. Following harvest, some slides were studied routinely, using GTG banding techniques, and two slides were prepared using standard protocols provided by cytose cell probe kit.

The diagnostic probe is designed for the q11.2 region of chromosome 22 and the telomeric region as an internal control. The presence of the probe on only one of the chromosomes 22 was compatible with a microdeletion in q11.2 band region, diagnostic for DiGeorge/Velo-cardio-facial syndrome. In analysis on standard karyotype a microdeletion of long arm of chromosome 22 was suspected in 550 and above band resolution, but was not conclusive.

CONCLUSION: Detection of microdeletion such as 22q11.2 band region can be suspected in routine cytogenetic analysis, but requires advanced molecular cytogenetic techniques for confirmation.

P0322. Cytogenetics findings in Iranian patients with repeated fetal loss

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Most series studies of couples with two or more fetal loss or stillbirths show 5-6% chromosomal aberrations. In an attempt to compare the data in our center with other centers, we did a retrospective study of the results of 1622 karyotypes of couples referred between 1997-2002 for two or more of the following, abortions, IUFDs, stillbirths, and perinatal death of offspring. Karyotypes of these couples were prepared after routine culture, harvest, spreading, and GTG banding techniques. In some cases C-banding and NOR analyses were also performed. 10-15 spreads were screened and 5-10 spreads were analyzed routinely under the microscope, and in cases of mosaicism up to 100 spreads were counted. Five microphotographs were taken and two karyotypes were done per case. The indications of referral were 898(57%) individuals for two or more abortions, 169 (6%) with IUFD, 166(16.3%) with perinatal death of children, 356(18%) with at least one abortion and another cause, and 33 (1%) with IUFD and dead child. The results indicate that chromosomal aberrations in couples with two or more abortions, abortion with other problems, dead-child, IUFD, IUFD with dead-child were 5.5%, 4.5%, 8%, 3%, 3%, respectively. Overall, 5.3% had chromosomal aberration, 72% of which were translocations, 17% inversions and 11% numerical translocation abnormalities. Balanced reciprocal translocations were the most frequent, while 10% had Robertsonian translocations. The results of this study are comparable with results from other centers and emphasize the necessity of chromosomal study in couple with history of fetal loss or death of non syndromic offspring.

P0323. Subtelomeric rearrangements detected in patients with idiopathic mental retardation: Results and a comparison between telomere Multi FISH probes and Multiplex Ligation-Dependent Probe Amplification(MLPA)

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Screening of subtelomeres with both telomere Multi FISH probes (Cytocell[®]) and MLPA (MRC-Holland[®]) was performed in 40 patients with idiopathic mental retardation, dysmorphism and/or congenital malformations.

FISH with multiple subtelomeric probes detected 5 patients (12.5%) with a subtelomeric aberration (Table I). In addition, 2 patients were found to carry a polymorphism in 2qter region. All of them were later confirmed by using complementary methods (specific telomere FISH probes and microsatellite analysis) and the origin of the restructuration was established.

Patient	FISH results	MLPA results Loss Gain	Parents
1	46, XY. ish der (2) t (2;10) (q37.3;q26.3) monosomy 2q/ trisomy 10q	2q 10q	Paternal t(2;10)
2	46, XY. ish del (2) (q37.3) monosomy 2q	2q	de novo
3	46, XX. ish der (1) t (1;22) (p36.3;q13.3) monosomy 1p/ trisomy 22q	1p 22q	de novo
4	46, XX. ish del (9) (q34.3) monosomy 9q	9q	de novo
5	46, XY. ish der (15) t (15;17)(q26.3;p13) monosomy 15q/ trisomy 17p	unde- tected 17p	unknown

The diagnostic capacity of MLPA (salsa PO36) to detect subtelomeric chromosomal abnormalities was tested by screening the same patients previously studied by FISH. MLPA found the same subtelomeric abnormalities in all cases except from patient 5, in which a 15qter deletion was misdiagnosed. Furthermore polymorphisms in 10qter were detected in 3 patients and confirmed afterwards.

MLPA is revealed as a cheaper, less time consuming technique than the previously reported methods for telomere screening. It could be a reliable technique to detect submicroscopic telomeric copy number changes, rendering it suitable for routine diagnostic screening in mentally retarded patients. Nevertheless, all chromosomal aberration detected should preferably be confirmed by independent methods.

In order to provide additional cases to the literature on subtelomeric abnormalities and their genotype-phenotype correlations, we present a summary with the main clinical features of patients in which a chromosomal abnormality was detected.

P0324. Deletion of distal Xp in a male newborn

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We present a case of a correlation between extremely low unconjugated estriol (uE3) and a deletion of distal Xp respectively deletion of the steroid sulfatase (STS) gene. Amniocentesis in 20-year-old woman was performed in the 21-week of gestation. It was based on the positive triple test (repeatedly low maternal serum uE3). In spite of identified normal karyotype 46,XY deletion of distal Xp was assessed by fluorescence *in situ* hybridization (FISH) and the same deletion was confirmed in mother. The pregnancy was complicated with polyhydramnion. The male-infant was born in the 32-week of gestation by Caesarean section (1490 g, micropenis, cryptorchismus). Chromosome analysis of peripheral blood lymphocytes proved 46,XY,del(X)(p22.3) karyotype, which was verified by FISH. The infant died after bleeding in central nervous system at the age of 43 days.

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P0325. Chromosome damage observed in blood samples submitted to cell phone radiation

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The impact of the cell phone technology and the increasing number of users all over the world have raised questions regarding cancer and other diseases etiology and time of exposure, cumulative exposure and age-related trends. Many reports specially out from the REFLEX study group (Europe) showed that the cell phone radiation, called non-ionizing radiation (NIR) might affect the DNA double-helix producing breaks and other alterations that cannot be repaired by the cell machinery. The object of this study was to investigate chromosome aberrations in blood samples submitted to NIR. Eight healthy donors were selected and serial blood samples were taken (total of 48 samples). The samples were cultured in a highly monitored setup that was designed for this project. Cells were submitted to an average power (SAR values) of 2W/kg (International Limit), 5W/kg and 10 W/kg in both AMPS and CDMA technologies. At least 200 cells per irradiation, per patient were analyzed. The sham-exposed and the patient controls showed a frequency of ZERO for chromosome aberrations. Both AMPS and CDMA technologies showed increasing frequencies of chromosome aberrations per cell according to SAR levels of 2, 5 or 10 W/kg. Acrocentric chromosomes satellite length alterations were also highly observed. Although the number of alterations was not high per cell we concluded that the radiation was able to produce DNA instability in our samples and that these findings should be taken into account on radiation public policies management and to establish safe limits of human exposure.

SUPPORT: FUNTTEL

P0326. The application of Array Comparative Genomic Hybridization in a routine cytogenetic lab: analysis of a familial duplication of chromosome 13q.

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A DNA microarray was used with a resolution of 0.6 Mb for the detailed analysis of a partial duplication of chromosome 13 in a family. Routine cytogenetic analysis (GTG 550 band level) revealed a partial duplication of chromosome 13q.

Six persons in three generations carry the duplication. They have mild mental retardation, behavioural problems, seizures, hearing loss, strabismus, dental anomalies, hypermobility, juvenile hallux valgus, and mild dysmorphic features.

In order to define the boundaries of the duplication and to get possible insight in the phenotype-genotype correlation we constructed a 0.6 Mb genomic microarray, based on the 1 Mb BAC clone set of the Wellcome Trust Sanger Institute. Array Comparative Genomic Hybridization showed the duplication to span approximately 21 Mb, ranging from chromosome band 13q21.31 to 13q31.1. The position of the breakpoints was confirmed by routine FISH.

The region involved harbors about 40 genes (Human Genome Browser UCSC, release may 2004), and an increased dosage of one or more of these genes may be responsible for the characteristic symptoms in this family members. No obvious candidate genes were found in evaluating function and expression patterns.

The relative mild presentation of this large duplication may be explained by the relative paucity of genes in the chromosome region involved. The use of small, chromosome specific microarrays in routine cytogenetics will allow a more reliable genotype-phenotype correlation in patients with chromosome abnormalities, when the number of data molecular (cyto)genetic and clinical data increases.

P0327. Analysis of aneuploidy frequency in uncultured and cultured cells from workers upon exposure by nuclear-chemical 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 (15 individuals) and clinically healthy men (10 individuals) was performed by use of two-color fluorescent *in situ* hybridization. The total value of numerical aberrations of all six chromosomes was similar in non-stimulated cells of both groups: $1.84 \pm 0.73\%$ and $1.96 \pm 0.99\%$ in control and exposed groups respectively ($P=0.73$). 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*. Frequency of aneuploidy of several chromosomes in the cultured cells of exposed workers has increased in comparison with control. Significant differences were found in the incidences of hypo- and hyperploidy of chromosome 12, hypoploidy of chromosome 11, and hyperploidy of X-chromosome. Intergroup difference between the total value of numerical aberrations of all the six chromosomes was found, although it was not significant: $2.26 \pm 0.93\%$ in control, $2.98 \pm 1.11\%$ in exposed individuals ($P=0.085$). Thus, stimulated cells themselves introduce basic contribution in intergroup differences of numerical aberrations. We suggest that the expression of accumulated *in vivo* premutagenic damages of spindle apparatus takes place during first cell division. These anomalies are manifested during cell division and result in numerical chromosomal aberrations.

P0328. Cytogenetic studies in 196 cases of myelodysplastic syndromes

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The myelodysplastic syndromes (MDS) are a group of disorders in which bone marrow dysfunction is caused by both qualitative and quantitative defects of the hematopoietic cells. From January 1997 to December 2001, 196 cytogenetic studies on bone marrow cells from MDS were performed in our centre. Based on the WHO classification the cases were distributed as follows: 5 (2.5%) Refractory anemias (RA), 14 (7%) Refractory anemias with ringed sideroblasts (RARS), 82 (42%) Refractory cytopenias with multilineage dysplasia (RCMD), 50 (25.5%) Refractory anemias with excess blasts (RAEB), 5 (2.5%) 5q-syndromes, 37 (19%) Chronic myelomonocytic leukemias (CMML), and 3 (1.5%) unclassifiable. A cytogenetic result was provided in 189 (96%) cases. Ninety six cases (51%) showed an abnormal karyotype, with a single chromosomal anomaly in 60 (63%) cases, two anomalies in 11 (11%) cases and a complex karyotype in 25 (26%) cases. The chromosomal anomalies more frequently found were: del(5q), trisomy 8, monosomy 7, del(7q), del(11q), del(20q), trisomy 21 and loss of Y. The MDS subtypes with a higher frequency of chromosomal abnormalities were RAEB (71%), RCMD (46%) and CMML (34%). The cytogenetic abnormalities defining a poor prognosis (based on the IPSS cytogenetic classification) were found more frequently in the RAEB (37%) and RCMD (14%) subtypes, being complex karyotypes in the vast majority of cases. Cytogenetic studies are an useful tool in the diagnosis, prognosis and follow-up of patients with a MDS.

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P0329. The role of fluorescence in situ hybridization (FISH) in genetic diagnosis of infertile men

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Chromosomal abnormalities often lead to infertility. Structural chromosomal disorders are detectable from peripheral blood by karyotyping and metaphase FISH. Carriers of reciprocal translocations have increased risk of a chromosomally unbalanced offspring. Using FISH with specific DNA probes we can determine the chromosomal segregation pattern of the translocated chromosomes in spermatozoa. In the other investigated group there are XX sex-reversed males, where the testicular differentiation can occur in the absence of the Y chromosome.

Results: We investigated the segregation pattern of the translocated chromosomes in two human male carriers of a reciprocal translocation. One of them had Y-3 translocation: t(Y;3)(q12;p21). The rate of genetically normal and balanced sperms in ejaculate were only 31%, while the rate of abnormal ones were 69%. The other patient had 1-17 translocation: t(1;17)(p17;q11). The segregation result showed that the genetically normal and balanced spermatozoa were 53.7%. We evaluated 3 XX sex-reversed patients by FISH analysis for SRY gene specific and X chromosome specific DNA probes. We detected SRY gene in each cases on the X chromosome which caused the male phenotype.

Conclusion: FISH is an informative technique for assessing the percentage of abnormal sperms in translocation carriers. These results ensure more accurate genetic counseling for patients in assisted reproduction centres. The testis determining factor (SRY) is localised on the X chromosome in our XX sex-reversed patients, but genes responsible for spermatogenesis are missing. In these cases there are not gametes even in the testis for fertilization, so donor insemination is the only way having children.

P0330. The first reported case of duplication/deletion mosaicism of the 7q(21.1>31.3) region

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Sunyer), Barcelona, Spain, ⁴Servei de Neonatologia, Hospital Clínic, Barcelona, Spain.

Mosaicism for structural aberrations is a rare event and the coexistence of a cell line with duplication and another with deletion of the same chromosome segment is even more infrequent. We report on a newborn male with a mosaicism 46, XY, dup(7q) / 46, XY, del(7q) in lymphocytes. The patient is trisomic for region 7q21.1 to 7q31.3 in 90% of analysed metaphases and monosomic for the same region in 10% of metaphases. He is the first child of a 31-years-old mother and was delivered during 32nd week of gestation by Caesarean section because of oligohydramnios and foetal tachycardia. The parents were unrelated and have normal phenotypes and karyotypes. The newborn presented pneumothorax and the main dysmorphic features were: microretrognathia, low and displastic ears, short neck, small mouth, cutaneous syndactyly on 2nd and 3rd toes, hip dislocation and long fingers with metacarpo-falangeal and interfalangeal articulations hyperextensibility. No other anomalies were observed. Due to the absence of a normal cell line, we propose that the mosaic is the result of an abnormal chromosome recombination in a very early embryonic mitotic division. Child clinical features and survival may be due to the high proportion of trisomic cells versus monosomic, and were compared with published cases of partial trisomy 7q and partial monosomy 7q. To our knowledge, no other patient showing mosaicism dup(7q)/del(7q) has been reported.

P0331. FRA18C, a new fragile site, possibly associated with in vivo chromosome breakage

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We are studying a patient with Beckwith-Wiedemann syndrome (BWS) characterized by overgrowth, loss of IGF2 imprinting and an 18q22.1 truncation. Interestingly the father of the patient expressed a hitherto unknown fragile site, FRA18C, on chromosome 18q22.1.

FISH experiments on metaphase spreads of the patient showed hybridization of an 18q subtelomeric probe only to the intact chromosome 18, indicating that the deletion in the proband is a pure terminal truncation. No abnormalities could be detected when the same FISH experiment was performed on metaphase spreads of the father, excluding the possibility that the de novo deletion in the proband is due to a balanced translocation or other chromosomal rearrangement. We cloned the chromosomal breakpoint and showed that the truncation was stabilized in vivo by the addition of repetitive telomeric sequence (TTAGGG).

The breakpoint region contained a lot of AT rich repeats, a feature of common fragile sites. We found these AT rich repeats to be polymorphic, but no expansion could be detected in the patient or its father. One of these AT rich repeats resembles the consensus sequence of a common fragile site. According to the computer program M-FOLD, this repeat sequence folds into a hairpin structure, another feature of sequences found in common fragile sites. Using the program Twistflex, we found evidence for the presence of flexibility islands in the breakpoint region. Together, these data suggest, for the first time, that a common fragile site may be involved in in vivo chromosome breakage.

P0332. Characterization of a der(18) in a female patient with multiple congenital abnormalities using several molecular cytogenetic techniques

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A one-month old girl, born after an uncomplicated pregnancy and delivery to healthy non-consanguineous parents, presented with failure to thrive and persistent respiratory insufficiency. There were feeding difficulties and protein losing diarrhea. Facial dysmorphic features were present, e.g. slight frontal bossing, high arched eyebrows and a flat nasal bridge. Her chest was narrow and asymmetrical. A cardiac murmur was detected, based on pulmonary stenosis. Cardiac hypertrophy was found without a detectable mutation in the *MyBPC3*-gene. At the age of one year the girl had developmental age of approximately six months.

GTG banding performed at the age of three months revealed a der(18). Fluorescence in situ hybridization (FISH) showed a partial deletion of the short arm of chromosome 18 and additional chromosomal material was present. The nature of this additional material was elucidated by spectral karyotyping (SKY) to be derived from chromosome 1. Array based comparative genomic hybridization (Array-CGH) was used to determine the size and location of both the deletion in chromosome 18 and the additional material of chromosome 1. The breakpoint in 18 was identified in p11.2, the additional chromosome 1 material was present from band q42.1 up to qter. A comparison with the literature will be presented.

P0333. Increasing complexity of a complex (1 ;4) chromosomal rearrangement

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Recently several articles have focused on a high degree of unexpected additional complexity observed using array-CGH to characterize breakpoints and to screen for cryptic imbalance in de novo chromosomal rearrangements. Here we report on an apparently four breaks rearrangement observed in a 2 years 8 months old boy presenting with language delay and poor communication. He had no facial dysmorphism excepted bilateral epicanthus and strabismus. A combination of classical cytogenetics and chromosome painting studies using WCP1 and WCP4 showed a rearrangement between chromosome 1 and chromosome 4. The segment distal to 4q28 was translocated to 1p32; the 1pter-p33 segment was divided in two parts, the more distal part was translocated onto 4q28 and the proximal one was inserted into 4q26. Parents karyotypes were normal. CGH-arrays was performed to screen for cryptic imbalance. A deletion of about 6 Mb was detected at 4q28.3 between BAC 401i19 and BAC 425j20 corresponding to the observed breakpoint on chromosome 4. This deletion was confirmed using FISH and metaphase-CGH. However the observation of a very dark G band in 4q28 on the der(4) was evocative of a duplication rather than a deletion. This inconsistency between CGH array and banding is puzzling. It could result from a duplication or an amplification not detected by the probes used for CGH-array or due to a change in chromatin structure. This suggests that still another type of complexity may be associated with de novo chromosomal rearrangements.

P0334. The incidence of acquired karyotypic aberrations in childhood ALL is influenced by presenting age and cell type

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Acute lymphoblastic leukaemia (ALL) represents the most frequent malignant disease in childhood and has a peak incidence between the age of two and six. It has been generally accepted that a cytogenetically abnormal clone will be found in 85-90% of all cases. Over a 10-year period we have karyotyped a consecutive series of 394 childhood ALL cases at presentation (aged 16 or under). Among these cases there were 355 with B-cell lineage and 39 of T-cell origin. In all cases a conventional cytogenetic result was obtained and in 364 (92.4%) the presence of an abnormal clone could be demonstrated. The remaining 30 had an apparently normal karyotype, but by employing a range of FISH probes (including TEL/AML, BCR/ABL, and HOX11L2) a further 7 were identified as having clonal chromosomal changes. Analysis according to age and cell type showed significant differences in abnormality rate, 95.5% of B-cell disease showed karyotypic changes, while an abnormal clone was identified in only 79.5% of T-cell disease (p-value = 0.0003). When age was considered 235/244 (96.3%) of children presenting below the age of 6-years showed a clonal chromosomal change, whereas only 134/149 (89.9%) of children over the age of 6 showed clonal changes (p-value = 0.019). In both age groups T-cell disease constituted one third of the cytogenetically normal cases. Therefore, these results indicate that a true normal karyotype is a rare event in childhood ALL, but its likelihood is influenced by age and cell type.

P0335. Interstitial deletion of 6q without phenotypic effect.

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Euchromatic interstitial deletions without detectable phenotypic abnormalities are rarely encountered. Identification of these variants is important for prenatal diagnosis and genetic counselling.

A 34-year-old woman with normal intelligence was referred for karyotyping because of recurrent abortions. With the exception of a bicuspid aortic valve without hemodynamic consequences, which is a common minor anomaly in the general population, no dysmorphic features were found on physical examination. Conventional chromosome analysis (GTG-banding) revealed an interstitial deletion in the long arm of chromosome 6. With array comparative genomic hybridization (aCGH) the size of the deletion was estimated to be between 9.9 and 11.6 Mb and the exact karyotype is 46,XX,del(6)(q22.31q23.1). Her son with developmental delay, behavioural problems and mild dysmorphic features has a normal karyotype and is not a carrier of the deleted chromosome 6.

P0336. A de novo complex chromosome rearrangement (CCR) involving chromosome 2, 13, and 14 in a chorionic villus biopsy

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Complex chromosomal rearrangements (CCR) are rare structural chromosome aberrations characterized by three or more breakpoints located on two or more chromosomes. We report on a healthy 43-year-old G3P1 woman who was referred to our hospital for a chorionic villus biopsy at 11 weeks of gestation because of advanced maternal age. Cytogenetic analysis of G-banded chromosomes demonstrated a complex rearrangement involving chromosomes 2, 13 and 14 in all cells of the short-term and long-term cultures. Fluorescence in situ hybridization (FISH), using specific probes for chromosome 2, 13, and 14, confirmed the complexity of the rearrangement and showed that the derivative 13 is composed of 5 distinct segments in the following order: 13q proximal, 2q, 13q, 14q, and 2q. The derivative 2 consists of the short arm of chromosome 2 and the distal part of 14q. The derivative 14 is derived from 14q and 13q. Parental chromosomes were normal. Based on FISH and cytogenetic results the karyotype of the *de novo* CCR involving 3 chromosomes with 6 breakpoints was written as: 46, XY,der(2)(2;14)(q1?3;q24),der(13)(13pter->13q1?3::2q?->2q?::13q?->13q?::14q?->14q?::2q?->2qter),der(14)(t(13;14)(q2?2;q2?2)). Counseling was offered to the patient and her husband. At 15 weeks of gestation the pregnancy was terminated. Postmortem examination of the fetus revealed a mild facial dysmorphism (tall forehead, short bifid nose with anteverted nares, retrorhinognathia, low-set ears) and no additional external malformations. Tissue culture of cartilage and cord cells confirmed the CCR. To determine whether the CCR is balanced, we have performed array-CGH (comparative genome hybridization) and results will be presented.

P0337. A comparative study on detection efficiency of classical cytogenetic and Interphase Fluorescence In Situ Hybridisation in diagnosis of mosaic form of X chromosome aneuploidies.

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Standard cytogenetics technique is of value in detection of chromosomal abnormalities. However the detection of chromosomal mosaicism is often difficult due to time constraints and limited number of available metaphase cells. Interphase fluorescence in situ hybridisation (FISH) can be utilized to study the number of copies of a specific chromosome in interphase cells. The technique has valuable application in detection of chromosomal mosaicism since, a minimum of 100 cells can be analysed in a limited time.

We employed the GTG-banding assay followed by interphase FISH and biotin labelled DXZ1 (Q-Biogene) to enumerate the X chromosome on peripheral blood samples of 88 women demonstrating the clinical features, compatible with X aneuploidies. Thirty-two samples were detected as 45,X and 3 samples as 47,XXX by GTG-Banding method

and confirmed by Interphase FISH. Nine Samples were detected as suspicious 45,X/46,XX and 2 samples as 45,X/46,XX/47,XXX by conventional cytogenetic studies and were subsequently confirmed by Interphase FISH. Five samples were diagnosed as normal by conventional cytogenetic studies, while 4 samples were shown to be mosaieic 45,X/46,XX and the fifth sample 45,XX/46,XX/47,XXX by interphase FISH analysis. Six samples showed structural aberrations of chromosome X, which were undetectable by Interphase FISH. The remaining 21 samples were revealed to be normal by both cytogenetic and FISH analysis.

The results indicate that interphase FISH is a useful and reliable tool in detection of mosaieic form of aneuploidies. However the technique has to be used as a parallel to conventional cytogenetic methods, which allows detection of structural chromosome abnormalities.

P0338. Familial dic(4;15)(p16;q11.1) associated with severe psychomotor retardation

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Translocation of 15q11 on to autosomes is a relatively common event, which has no adverse clinical consequence. We report here a case of familial dic (4;15) associated with a severe phenotypic effect. A female neonate who presented with convulsions and a cardiac defect showed a satellited chromosome 4 (46,XX,4ps pat), inherited from her phenotypically normal father. She developed severe psychomotor retardation and epilepsy.

The identification of autosomal non-acrocentric breakpoints and the acrocentric donor is often difficult. Combined use of conventional and molecular cytogenetics allowed the identification of the unbalanced translocation 46,XX,4ps pat.ish+15,dic(4;15)(p16;q11.1)(D15Z1+, D15S10-, SNRPN-, 4p-, D4Z1+). By FISH the satellited chromosome 4 in the proband and her father was positive with centromere 15 and 4, and negative with probe for Prader/Willi Angelman region and telomere 4. Future molecular characterisation is therefore needed in order to determine a small tandem duplication of DNA within the subtelomeric region, which may have been missed in our case. There is evidence that small chromosomal rearrangements involving the terminal bands of chromosomes are an important unrecognised cause of mental retardation in inherited rearrangements.

P0339. Two cases of de novo aberrations of 7q

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De novo chromosomal aberrations represent a considerable risk to their carriers; even in cases of balanced forms they can result in an abnormal phenotype.

The first patient is a 6.5-year old boy who, in contrast to his twin brother, has ectrodactyly affecting both hands and feet (SHSF). This phenotype can occur as an isolated anomaly or as a component of multisystem syndromes. The SHFM1 locus was mapped to chromosome 7, band q21.2-22.1 and SHSF has often been described in patients with deletion or duplication of 7q. In our patient we detected a *de novo* paracentric inversion 46,XY,inv(7)(q21.2q36). His brother's karyotype is normal. The mother was found to be a low frequency mosaic for trisomy X.

The second case is a 2-year old girl with lip-palate-maxilla cleft, facial dysmorphology and mild-to-moderate PMR. Considering that the karyotype contains two *de novo* chromosomal aberrations 46,XX,t(1;1)(p31.1;q21),del(7)(q31.3q34), the girl is relatively mildly affected.

P0340. Chromosome anomalies in infertile males

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716 infertile men were studied with different types of spermatogenesis disorders for determining type and frequency of chromosome anomalies. In all patients sperm analyses and cytogenetic investigations were provided. From the total number 416 men was diagnosed as an azoospermic and 300 as an oligozoospermic. In the azoospermia

group (416 males) chromosomal anomalies was found in 90 patients. More frequent was sex chromosome anomalies (78 patients -86,7%), structure of chromosomal anomalies was following: 47,XXY- in 64 patients (71,1%), 46,XY/47,XXY- in 8 patients (8,9%), 46,XX/47,XXY- in 1 patient (1,1%), 47,XYY- in 1 patient (1,1%), 46,XY/47,XYY- in 1 patient (1,1%) and 45,X/46,XY- in 1 patient (1,1%). Y chromosome macrodeletions was detected in 4 patients (4,4%), 46,X,delY(q11). Autosomal translocations was found in 3 patients (3,3%). In 2 cases (0,5%) karyotype was 46,XX.

In oligozoospermia group (300 males) chromosomal anomalies was detected only in group of patients with severe oligozoospermia (159 patients-53%). In 10 cases (6,3%) chromosomal disorders were found. In 7 cases (4,4%) sex chromosome abnormalities was shown with the following karyotypes: 47,XXY- (1), 47,XYY- (1), 46,XY/47,XXY- (4) and 45,X/46,XY- (1). In 3 cases (1,9%) autosomal translocations was detected.

The FISH method detects SRY gene translocation on X chromosome in the patients with the karyotype 46,XX.

Cytogenetic investigation considered as very important to detect the cause of infertility. It is also recommended to perform IVF, ICSI, TESA techniques with the use of preimplantation prenatal diagnostics in the families of infertile men with chromosome anomalies.

P0341. Molecular analysis of Y-chromosome in XX-males

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XX-male syndrome is rare sex determination abnormality characterized by bilateral testes development in absence of a cytogenetically identifiable Y chromosome.

To analyze presence of SRY gene and other Y-chromosome loci we investigated cohort of 19 phenotypic men with karyotypes: 46,XX (n=15), 45,X/46,XX (n=3) and 45,X (n=1).

Molecular analysis was performed on leucocytes DNA by amplification of SRY, AMG/AMGL, ZFX/ZFY and seven Yq loci: sY84, sY86, sY615; sY127, sY134; sY254, sY255 in two multiplex PCR. Breakpoint mapping was carried out for SRY-positive patients by analyzing of following STSs: sY2062, sY1248, sY211; sY1240, sY716, sY1241, sY1219, and sY1209.

We found SRY gene in 15 (79%) of 19 patients, whereas AMGL and Yq loci were absent in all cases. SRY gene was found in all three 45,X/46,XX males and one of two 46,XX males with incomplete masculinisation. Breakpoints localized near proximal to interval 1A1B (class 1 of (Y+)XX males) were revealed in 3 patients including 45,X male. Yp fragments containing intervals I-II (class 2) and I-III (class 3) were found in four and eight SRY-positive men, respectively. It is significant that sY1219 and sY1209 were disclosed in all of class 3 (Y+)XX males. It may be explained by Yp paracentric inversion. Obtained data confirmed that about 80% XX-males are SRY-positive, as well as the size of Yp fragment was not correlating with the degree of masculinisation. Class 3 represents the most common form of SRY-positive XX males resulting from Xp-Yp translocation. Yp inversion predisposes to abnormal X-Y interchange.

P0342. Sperm segregation analysis of 4 rare Robertsonian translocations

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Analysis of chromosomal segregation in sperm of Robertsonian translocation carriers is of great interest for understanding the mechanisms of translocation transmission and for assessing the risk of conceiving chromosomally abnormal children. To date, most of the sperm segregation studies of Robertsonian translocations have been performed on the most common rearrangements : t(13 ;14) and t(14 ;21). In the present study, meiotic segregation has been analysed in sperm from 4 males heterozygous for rare Robertsonian translocations, i.e. t(13 ;15), t(13 ;22), t(14 ;22) and t(15 ;21).

After decondensation, sperm was analysed by dual color FISH, using

either locus-specific probes, or whole chromosome painting probes. A mean of 6,000 sperm nuclei was scored per patient (ranging from 4,300 to 9,000). The frequency of normal and balanced spermatozoa ranged from 86% to 91%. The incidence of unbalanced complements resulting from adjacent segregation accounted for 9% to 13.5%. When compared, both locus-specific probes and whole chromosome painting probes gave similar results for segregation patterns, demonstrating the efficiency of the 2 procedures for sperm study in Robertsonian translocation carriers.

This study shows that rare Robertsonian translocations display segregation patterns identical to the common Robertsonian translocations previously analysed. These data support the existence of similar meiotic behaviour for all the nonhomologous Robertsonian translocations.

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P0343. De novo 2q3 deletion in female patient presenting with syndromic cleft palate

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We report a 3-month-old female patient presenting a de novo interstitial deletion of the long arm of chromosome 2 [46,XX,del(2)(q32.2q34)] identified on a 400 band chromosomal analysis. The antenatal ultrasonography revealed intrauterine growth retardation and oligoamnios at 19 and 36 weeks of gestation respectively. The child was born at 39 weeks of gestation (birth weight 2,865 g, crown-heel length 47.5 cm, head circumference 33 cm). She presented with a weak cry, failure to thrive, severe hypotonia, and psychomotor delay. The facial features associated down-slanting palpebral fissures, hypertelorism, sloping forehead, poorly folded helices, low-set and posteriorly rotated ears, large nose, cleft palate and micrognathia. Cardiac ultrasonography revealed aortic root dilatation. Cranial ultrasonography showed a short corpus callosum without other morphological abnormalities. Abdominal ultrasonography and skeletal radiography were normal. Parents' karyotype was normal. Molecular analysis showed that the deletion occurred on the paternal allele, and was flanked by the D2S364 and D2S147 markers, defining a 23-Mb region containing at least 70 genes. The *SATB2* gene, flanked by the deleted D2S318 and D2S311 markers, has already been implicated in isolated cleft palate. The *COL3A1* and *COL5A2* genes, encoding type-III procollagen and alpha2 chains of the type-V collagen respectively, are also located in this region. The *COL3A1* gene is involved in vascular Ehlers-Danlos syndrome (type-IV) with a risk of arterial dissection. The involvement of *COL5A2* in classic Ehlers-Danlos syndrome is still controversial. Interstitial deletions of the middle portion of chromosome 2q are rare; clinical and molecular characterisation may allow to draw some genotype-phenotype correlations.

P0344. Subtle subtelomeric rearrangements in children with developmental delay

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The prevalence of mental retardation (MR) is 1-2% in general population, and has serious implications for the affected individual, the family and society. In course of diagnostic management of the patient special attention is paid to genetic investigations. Chromosomal aberration is the most common cause of mental retardation and present in 4-28% of affected individuals. The traditional cytogenetics is however unable to detect genomic abnormalities smaller than 5-10Mb. These small submicroscopic changes of genetic material can be detected by molecular cytogenetic methods. 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. This investigation included 31 child 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 multicolour FISH probe panel ToTelVysion (Vysis). Aberrations of subtelomeres were detected in 2 (6.4%) of patients. Our results point out the usefulness of FISH method

for screening subtelomeric regions and present additional evidence that subtle subtelomeric aberrations have important role in the aetiology of mental retardation. Ministry of Science Croatia supported this work (TP-01/072-01).

P0345. Delineation of a 5q35 microdeletion in congenital heart disease: haploinsufficiency of NKX2-5 can cause Ebstein anomaly

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Congenital heart diseases (CHD) represent the most common group of inborn malformations, with an incidence of almost 1%. One of the genes associated with CHD is the *NKX2-5* gene, which encodes a cardiac homeobox transcription factor. Mutations of *NKX2-5*, which is located at 5q35.1 have been identified in a subset of familial and sporadic cases of atrioventricular (AV) conduction block associated with a spectrum of structural heart defects, including atrial septal defect (ASD) and tetralogy of Fallot. Functional studies of *NKX2-5* mutations have indicated that haploinsufficiency determines the AV conduction block and ASD phenotypes. However, some studies suggest dominant negative effects and it has not been possible to correlate other *NKX2-5* phenotypes with a specific mechanism of the mutation due to a small sample size.

Here, we present delineation of a microdeletion in a patient with AV conduction block, ASD and tricuspid valve malformation (Ebstein anomaly) in addition to microcephaly, scoliosis and pectoral hypoplasia. FISH mapping of an apparently balanced inversion, 46, XY, inv(5)(q13q35)de novo, revealed a 2.2 megabase deletion at the distal inversion breakpoint. This region contains 17 RefSeq genes, including *NKX2-5*. This case gives therefore strong evidence for haploinsufficiency of *NKX2-5* as the cause of Ebstein anomaly. Microdeletions in the terminal part of 5q are rare and only 12 cases have been published so far. A genotype-phenotype comparison with previous cases will be presented.

P0346. Cytogenetic Findings in Chorionic Villi of Spontaneous Miscarriages

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A proportion of spontaneous miscarriages are caused by chromosomal abnormalities. The aim of this retrospective study was to evaluate the contribution of chromosomal abnormalities in spontaneous abortions at 6 -13 weeks of gestation. We report cytogenetic findings seen in chorionic villi samples of 42 miscarriages from August 2001 to May 2004 in Mother and Child Health Care Institute of Serbia „Dr Vukan Cupic“.

Cytogenetic analysis was performed using direct method for chorionic villi chromosome preparation, and chromosomes were identified by standard banding techniques. According to the sonographic findings, these spontaneous abortions were characterized as blighted ovum and missed abortion.

Chromosome analyses of GTG banded metaphases showed normal karyotype in 18 cases (42.86%). Abnormal karyotypes were found in 24 cases (57.14%). 22 abortions had numerical aberrations: 13 were autosomal trisomies, one was autosomal monosomy, 3 were Turner syndromes (45,X), one was Klinefelter syndrome (47,XXY) and 4 were polyploidies (triploidy (69) and „near“ diploidy (46±)). Among autosomal trisomies, extra chromosomes 4, 6, 13, 16, 20 and 22 were present. In three abortions structural chromosome abnormalities were found: one unbalanced t(10;18)(p13;q23) translocation and one deletion 18q21. In our sample of spontaneous abortions, there were 11 blighted ovum abortions and 31 missed. The highest frequency of abnormal karyotypes was found in the cases of missed abortions. Cytogenetic investigations of spontaneous abortions provide valid informations as to the cause of abortion and contribute to prenatal diagnosis in subsequent pregnancies.

P0347. A complex chromosomal rearrangement involving chromosomes 10, 11 and 12: Cytogenetic, Microarray and Clinical characterisation.

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A report of a *de novo*, complex chromosomal rearrangement involving three chromosomes with four breakpoints. The karyotype was established by GTG banding, and WCP: 46,XY,t(10;12;11)(10qter-10p11.2::12q12-12qter;10pter-10p11.2::12q12-cen12-12p12::11p14-11pter;11qter-11p14::12p12-12pter). A chromosome translocation was first diagnosed at amniocentesis for increased serum biochemistry tests and mild Intra Uterine Growth Retardation. This was reported as an apparently balanced t(10;12)t(p11.2;q12) and the family were given a 5% risk of significant physical or intellectual problems. During the neonatal period the patient presented with hypothermia and hypoglycaemia. Weight and head circumference were on the 3rd centile. Repeat karyotype revealed that the chromosomal rearrangement was more complex than initially thought, involving a third chromosome, chromosome 11. As the patient got older it was evident that he had severe developmental delay, hypotonia with fine and gross motor coordination problems, short stature, severe visual impairment and sleep disturbance. Moderate facial dysmorphisms were also present (prominent nasal bridge, upslanting palpebral fissures, bushy eyebrows, widely spaced teeth). In addition, the patient had bilateral sacral pits, flat feet and a small penis. MRI showed hypoplastic optic nerves with a small optic chiasm. There were no other midline abnormalities. Initial microarray studies have been initiated and are currently being validated by FISH to determine whether the rearrangement really is balanced. Data will be presented.

P0348. Microduplication 22q11.2 syndrome : a cause of cognitive and behavioural problems - report of 3 familial cases

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Microduplications of the 22q11.2 region have only recently been observed examining interphase cells by FISH with TUPLE1 in patients referred for DG/VCFS (Ensenauer et al., 2003): they found a duplication in 1.5% of unrelated patients. To verify this finding a prospective study on 200 cases referred for DG/VCFS was performed, in which one patient (0.5%) with three copies of TUPLE1 was found. Since then a few patients with microduplication 22q11.2 were described, showing variable clinical phenotype ranging from mild learning disability to the presence of severe congenital malformations, or some overlapping features with DG/VCFS.

We report on a 8-year-old boy who was sent to us because of psychomotor retardation and behavioural problems. The parents were non-consanguineous. There was a family history of mental retardation (both parents had special education) and the father also had behavioural problems comparable to his son. There was no family history of cardiopathy. The standard karyotype of the patient was normal 46,XY as well as fragile X syndrome screening. FISH analyses 22q11.2 were performed and showed the presence of three signals for TUPLE1 probe in all interphase nuclei. FISH 22q11.2 analysis in the father and in one other brother disclosed the same microduplication. Complementary investigations showed that the patient had, in addition to his developmental delay and behavioural problems, asymmetric perceptive hearing loss and a small right kidney.

We'll discuss the clinical phenotype of these patients and compare it with literature data.

P0349. Molecular cytogenetic characterization of the mouse cell line WMP2 by spectral karyotyping (SKY) and multicolor banding (mcb) applying murine probes

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The Moloney murine leukemia virus-transformed suspension cell line WMP2 is derived from wild mice (*Mus musculus*) of the WMP/WMP strain. These mice carry nine pairs of metacentric Robertsonian translocation chromosomes. As the chromosomes of the wild-type mouse are all acrocentric, metaphase spreads of the WMP2 cells seem to be highly suited for physical gene mapping. Here we studied the WMP2 line using spectral karyotyping (SKY) combined with new established mouse specific multicolor banding (mcb) probes for the chromosomes X, 3, 4, 6 and 18. SKY revealed that the WMP2 cell line developed further four derivative chromosomes. After application of mcb five previously unrecognizable intrachromosomal rearrangements with 9 breakpoints were detected for the studied chromosomes: a translocation-chromosome including parts of the X-chromosome could now be described as der(9)t(9;X)(?;C); mcb 4 revealed in the dic(4;6) a deletion and an inversion in those two chromosomes; mcb 18 proved the presence of two dic(7;18) and a dic(8;8)(t(8;8;18) and of two different variants of the dic(7;18). Supported in parts by the DFG (436 RUS 17/49/02 and 436 RUS 17/135/03), the INTAS (2143), and the Deutsche Krebshilfe (70-3125-Li1). The cell line WMP2 was kindly provided by Dr. M. Rocchi (Bari, Italy) and Dr. H. Hameister (Ulm, Germany).

P0350. 2q Deletion: an unusual association of classic phenotype (Albright Hereditary Osteodystrophy 3) with hypothyroidism, epilepsy and multiple bone fractures

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Albright Hereditary Osteodystrophy 3 (AHO3) is a rare condition characterized by: short stature, stocky build, round face, mental retardation, brachymetaphalangia and eczema, associated with a deletion of chromosome 2q (2q37.3-qter). In this condition, soft tissue ossification and obesity are absent and there are no abnormalities in parathyroid hormone and Gs-alpha levels, unlike AHO1 and AHO2. We describe a case of a 37 years old woman with mental retardation, obesity (76 kg), normal stature (162 cm), slight dysmorphisms (roundish face, frontal bossing, detached earlobes, big and globose tipped nose with short pinnae, smooth philtrum, thin lips, short neck), hand and foot abnormalities, brachydactyly type E, hypothyroidism, epilepsy (normal EEG), and cutaneous eczema.

She has had 7 fractures, up to the age of 20 years (2 at wrist, 2 at ankle, left elbow, 2 at tibia and left perone) without major trauma, and epyphisis at right knee at 9 years. Calcemia, calciuria, RMN and cerebral TAC are normal.

Cytogenetic analysis showed a normal female karyotype, while subtelomeric probes revealed a deletion of the long arm of chromosome 2 (breakpoints under study). The karyotype was: 46,XX, ish del(2)(qter)2qtel-).

This evidence underlines the importance of the terminal region of chromosome 2 in the determining the AHO3 phenotype. The symptoms present in our patient could be explained by a breakpoint different to that described in the literature.

P0351. Recurrent 22q13.3 cryptic deletion in two brothers with clinical signs resembling the Clark-Baraitser syndrome phenotype.

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A preliminary diagnosis of Clark-Baraitser syndrome was performed in two brothers affected with a multiple congenital anomalies/mental retardation (MCA/MR) syndrome, born to healthy parents. They presented with moderate to severe MR, a psychotic personality, obesity, macrocephaly, a characteristic face with square forehead, prominent supraorbital ridges, bulbous tip of nose, short philtrum, gap between upper central incisors, large and coarse ears. Genitalia were normal. They also presented with big hands and feet and advanced bone age. One of them was excessively tall. On brain MRI, lateral ventricular dilatation with cortical and cerebellar vermis hypoplasia

was diagnosed. Chromosomes were apparently normal male. Although clinical signs were well consistent with the Clark-Baraitser syndrome phenotype, a search for subtelomeric cryptic rearrangements was performed by FISH, according to a general diagnostic policy in familial mental retardation. An isolated 22q13.3 cryptic deletion, spanning about 3.5 Mb, as established by additional FISH analyses, was diagnosed in both patients. Parents had normal 22q telomeres on 200 metaphases. Relevant considerations are the following: 1) a cryptic 22q13.3 deletion should be checked in patients with Clark-Baraitser syndrome phenotype; 2) the 22q13.3 deletion syndrome is distinctively associated with overgrowth and macrocephaly; 3) gonadal mosaicism is to be considered in genetic counselling of microdeletion syndromes.

P0352. Effect of F cells in the protection of cultured lymphocytes against DEB-induced chromosome breakage. The influence of GST activity and individual *GSTT1* and *GSTM1* genotypes

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Red blood cells (RBC) from normal adult individuals can protect cultured lymphocytes against chromosomal breakage induced by diepoxybutane (DEB), and the role of the polymorphic enzyme glutathione S-transferase T1 (*GSTT1*) (expressed in RBC) in the protective effect was already described. In the present work, we studied the influence of RBC extracted from umbilical cord blood of neonates (F cells) on the frequency of DEB-induced chromosome breakage in lymphocyte cultures from normal individuals. Simultaneously, we determined the total GST and catalase activities of RBC from controls and neonates, and individual *GSTT1* and *GSTM1* genotypes. Seven control RBC and six neonate F cell samples were used in the study. Phytohemagglutinin (PHA) stimulated lymphocyte cultures were incubated in the presence or absence of DEB (0.1 µg/ml), with addition of autologous RBC or F cells. The obtained results showed that, although both types of RBC protected cultured lymphocytes from normal individuals against chromosome breakage induced by DEB, the effect elicited by F cells was significantly higher, although variability in the protective effect among individuals was observed. It was also observed that the higher protective potency by F cells can be correlated with an increase in the total GST activity but not with catalase activity; concerning the individual variability in the protective effect, the correlation with *GSTT1* and *GSTM1* genotypes was demonstrated.

P0353. Untreated growth hormone deficiency with extreme short stature, cleft lip-palate and mental retardation in a 26-year old man with a de novo unbalanced translocation t(1;12)(q24;q24).

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We report on a 26-year-old patient presenting with extreme short stature (height 76 cm, weight 6.5 kg, OFC 42.5 cm) with short hands, facial dysmorphism with hypertelorism, beaked nose, cleft lip-palate and severe mental retardation. He was the unique child of non consanguineous parents. Measurements at birth were 43 cm for length and 2100 g for weight. He had severe feeding difficulties requiring enteral nutrition until the age of 3 years. Endocrine studies for severe growth retardation revealed severe growth hormone deficiency, but the child was untreated because of associated mental retardation. At 26 years of age, he could not walk or speak and had no puberty. Reinvestigations showed combined pituitary hormone deficiency, spondylo-epi-metaphyseal dysplasia with severe osteoporosis, enlarged aorta when indexed to the patient's size and normal cerebral MRI with apparently normal pituitary development. Conventional cytogenetic analysis revealed an apparently balanced de novo translocation t(1;12)(q24;q24). High resolution karyotype showed a 1q24-q25 deletion and comparative genomic hybridisation studies confirmed the 1q interstitial deletion. FISH studies of the both breakpoints using PACs and BACs permitted to further characterise the 1q interstitial deletion (1q24.2 to 1q25.2) and also revealed a

12q24.31 interstitial microdeletion. This observation is of interest for two reasons. First, these deletions could be a clue in the search of a gene responsible for growth hormone deficiency/midline defects. Second, it shows the importance of molecular cytogenetics in the study of de novo apparently balanced translocation with abnormal phenotype.

P0354. The 9p+ variant is an amplification of a repeat mapping within 9p21, and not a simple 9p11.2-p13.1 duplication.

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Among polymorphisms of chromosome 9, additional C-Band negative material on the proximal 9p have been rarely reported. They were described as duplications of 9p11.2-p13.1.

We report on three unrelated subjects with a chromosome expansion involving the proximal euchromatic region of chromosome 9p: a female, referred because of POF, subsequently ascribed to a premutation of the FMR-1 gene, and two adult, healthy males, who were referred after a prenatal diagnosis of 9p+ in the fetus. An unusual chromosome 9, with an additional homogeneously staining segment on the proximal 9p, was detected by conventional cytogenetics.

The additional region was R- and C-negative. Molecular cytogenetics was then performed by using a total of 5 molecular probes spanning the 9p11.1p13.1 region. We found that the euchromatic 9p+ polymorphism is caused by a great amplification (and not by a simple duplication) of an about 1.5 Mb repeat mapping within 9p12. This repeat is identified by BACs RP11-15E1, proximally, and RP11-402N8, distally, with a ratio RP11-15E1:RP11-402N8 of 2:1. The copy number of the repeat varied between 7 and 14 in individual cases. Based on these observations, we conclude that the 9p+ variant is a 9p12 amplification, and not a 9p11.2-p13.1 duplication, as suggested. This heteromorphism is not associated with clinical abnormalities. Important insights are achieved for the prenatal diagnosis of either a 9p+ variant, or a benign 9p-derived extra small chromosome.

P0355. Genetic counselling in Tunisian patients with reproduction failure having balanced chromosome structural abnormalities

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Carriers of balanced chromosome structural abnormalities are usually ascertained because infertility, recurrent miscarriages, failure of assisted reproductive technologies (ART), or offspring with unbalanced karyotype and multiple congenital defects.

Here we report 13 balanced chromosome structural abnormalities ascertained by cytogenetic analysis of 43 couples and 180 infertile men:

inv(9)(p12q12) in two men among couples with miscarriages

inv(10)(p15q21) in a woman with ART failure because absence of cleavage

Reciprocal translocation in two cases: t(7;15)(p22q25) and t(1;4)(q34;q26) in 2 women with respectively 5 and 3 spontaneous abortions

inv(12)(p12p12) in a man of a couple with early abortions after ART.

For the 180 patients with male infertility, we found 4 robertsonian translocations and 3 reciprocal translocations:

t(13;14) was found in two men with severe oligozoospermia and one azoospermic man

t(14;21) was detected in an oligospermic man

Reciprocal translocations were found in 2 azoospermic man [t(10;13)(q21;q22) and t(16;22)(p11;p11)] and one oligospermic man: t(4;9)(p15.3;p21).

In these cases, the central concept in genetic counselling is the estimation of the probability of unbalanced progeny at birth and other unfavourable pregnancy outcomes. More ever, the prognosis of success of ART is also an important concern because poor embryo development, which may be a result of high segregation abnormalities, may negatively affect the outcome of ART.

Estimates of unbalanced progeny at birth and the probability of poor embryo development are made on basis of theoretical and empiric meiotic segregation patterns but these risk estimates varied considerably from translocation to translocation and studies are often limited.

P0356. Diagnosis of subtelomeric imbalance using MLPA

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Subtelomeric imbalance is an important cause of idiopathic moderate/severe mental retardation. Established diagnostic procedures for detection of subtelomeric imbalance use multiprobe fluorescence in situ hybridization (FISH), which is labour-intensive and expensive, and therefore only applied for a subset of patients. In contrast, multiplex ligation-dependent probe amplification (MLPA) can be used to detect aberrant copy number at up to 50 loci in a single reaction.

A validation study of 271 cytogenetically normal and abnormal samples was carried out using commercial MLPA kits (MRC-Holland). Strategies for data analysis and interpretation were assessed with respect to false negative and positive rates. All known abnormalities were detected. A total of 23 samples with apparently terminal deletions on G-banded chromosome analysis were tested using MLPA; in three cases (13%) duplication of material from a different chromosome was identified, confirmed by FISH analysis as present on the abnormal chromosome. In 1 of the 23 cases the deletion was found to be interstitial, the MLPA probe being present in normal copy number. In addition, three cases were tested where G-banded chromosome analysis had found a derivative chromosome containing material of unknown origin. In all three cases the unknown material was identified; these results were confirmed using single FISH probes.

MLPA has now superseded FISH-based subtelomere screening in our laboratory. All positive results are followed up using single-probe FISH. As a consequence we are expanding our test population and have reduced reporting times. Service strategy and results will be discussed.

P0357. Incidence of 22q11.2 deletion syndrome in a selected population

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Almost all cases of DiGeorge syndrome or velo-cardio-facial syndrome result from a common deletion of chromosome 22q11.2. These individuals have a wide range of anomalies such as: congenital heart disease, particularly conotruncal malformations that are associated with 29% of deletions, palate abnormalities, hypocalcemia, immune deficiency, learning difficulties and characteristic facial features.

This syndrome may be inherited as an autosomal dominant trait. About 93% of patients have a *de novo* deletion and 7% have an inherited deletion.

Our goals were to assess the frequency of the deletion in a selected population of individuals with specific clinical features, mainly congenital heart defects, and the incidence of a familial transmission. A total of 325 individuals (251 infantile, 40 parents and 34 pregnancies) were evaluated by FISH (*fluorescence in situ hybridisation*) using specific DNA probes (N25 Vysis and TUPLE1 Qbiogene). Twenty of the 285 probands were identified with the deletion. Facial features are present in all of these cases and congenital heart defects are present in 85%, of these, 65% have a conotruncal defect. Three individuals with the deletion did not have any cardiac defect, only showing immune deficiency and hypocalcemia. This supports the need to be aware of less frequent features such as hypocalcemia. In our population 100% of the deletions diagnosed were *de novo*.

P0358. Rho gene expression regulates chondrocytic differentiation of mesenchymal precursor cells: a mechanism of TGF-beta/BMP2 in cartilage formation

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Mesenchymal precursor cells (MPC), an alternative source to provide

pluripotent precursors, have been recognized to give rise to osteocytes, chondrocytes, endothelial, adipose, and muscle cells. The previously reported results indicate that MPC chondrogenesis depends on both TGF-beta 3 and BMP2 signals. The *in vitro* data have shown that chondrocytic-like cells can be directly derived from fibroblast-like MPC after TGF-β3 and BMP2 treatment. However, during the chondrocytic differentiation, the mechanism of these growth factors regulates MPC morphological and genetic changes remains to be elicited. The TGF-β3/BMP2 regulated MPC genes that initiate and govern final cell phenotype remain unknown. To gain molecular understanding of MPC chondrogenesis, gene expression profiles of MPC treated with or without TGF-β3 and BMP2 were analyzed using cDNA microarray representing 11000 mRNAs from the human fetal liver isolated SH2/SH3/CD29/CD140+ cells (CD34/CD117-). The significant alterations in RhoA and RhoA-Rho kinase (RAK) gene expression levels were confirmed using RT-PCR. Genes that differently expressed in collagen II/ proteoglycan positive cells were identified. Compared with non-TGF-β3 or BMP2 treated cells, both RhoA and RAK genes were up-regulated respectively (4 to 7 times respectively). We also found that both collagen II and proteoglycan gene expressions increase following Rho/RAK expression. The up-regulated RAK signal transduction related genes such as DOCK180 and Rac were also detected. Inhibition of the RhoA and RAK mRNA expression with anti-RhoA/RAK antisenses significantly suppresses the MPC chondrogenesis. This data suggest that the RhoA/RAK signaling pathway is critical for MPC differentiation, which will require further functional testing.

P0359. Screening for cytogenetic and molecular chromosome rearrangements in children with conotruncal heart defects and supravalvular aortic stenosis

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Conotruncal heart defects and supravalvular aortic stenosis are the most cardiovascular malformations that have been associated with chromosomal microdeletions 22q11.2 and 7q11.2 respectively.

To estimate frequency and investigate clinical features of these microdeletions in unselected patients with heart conotruncal defects and supravalvular aortic stenosis, a total of 35 patients originate from the south of Tunisia are evaluated prospectively by cytogenetic and molecular studies.

The clinical analysis was performed according to a specific clinical protocol for the diagnosis of congenital cardiovascular malformations. All patients harbouring truncus arteriosus, interrupted aortic arch, tetralogy of fallot with or without pulmonary valve atresia, tetralogy of fallot with absent pulmonary valve, ventricular septal defect with malalignment of the conal septum and supravalvular aortic stenosis, were included in our study.

Cytogenetical analysis with RGH and GTG banding was used to detect chromosome rearrangements. All patients have normal karyotype 46,XX or 46,XY.

Molecular analyses were undertaken by fluorescent *in situ* hybridization using three probes: LSI DiGeorge N25 (D22S75) region probe N25/ARSA, LSI DiGeorge/VCFS region probe TUPLE1/ARSA and LSI Williams syndrome (elastin gene) region probe D7S486/D7S522. Results will be discussed and compared with those of other studies performed in the north and the centre of Tunisia.

P0360. Can the amplification of MSRV pol sequence be a marker of multiple sclerosis?

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Multiple sclerosis (MS) is one of the most frequent disorders of central nervous system characterized by demyelination foci in the brain and spinal cord. Pathogenesis of MS is poorly understood. Among etiologic factors some viruses are suggested to play important role.

The aim of our studies was the assessment of MSRV pol sequence copy number in MS patients compared to control individuals. Analysis was

performed on interphase nuclei and chromatin fibers from peripheral blood cells of 56 patients with MS, 8 patients with myasthenia and 20 healthy individuals.

For analysis of MSRV pol sequence copy number in the examined material the FISH with biotinylated PCR product was used. Detection of MSRV pol probe was carried out by reaction with avidin-fluorescein and biotinylated anti-avidin. MSRV pol sequences were found in all examined persons. However, the copy number of MSRV pol sequence was significantly greater in MS patients than in myasthenia and normal individuals. In addition, the MSRV pol sequence exists as tandem repeats on various chromatin fibers. The increased number of MSRV pol sequence has been found on chromatin fibers of MS patients as compared to myasthenia and healthy controls. This finding suggests that MSRV may play some role in pathogenesis of MS.

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P0361. Trisomy 4 p syndrome: clinical similarities with de Lange syndrome

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Trisomy 4p is a rare type of chromosomal disorder. Most of reported cases were originated from parental chromosomal rearrangements. Some of reported cases discussed as the similarities between this syndrome and Cornelia de Lange syndrome. Some of the authors claimed that there is a chromosomal locus related with de Lange syndrome while the others did not support this theory. We are here reporting a new case with partial trisomy 4p.

27 years old pregnant woman referred to our hospital as increased risk in triple screening test and history of a terminated pregnancy due to neural tube defect. Ultrasonographic examination was normal. Amniocentesis was offered and was performed due to triple test findings. Karyotype of the fetus was revealed an additional chromosomal material on chromosome 3. Chromosome analysis of parents presented maternal reciprocal balanced translocation 46,XX,t(3;4)(p26;p13) and fetus was diagnosed as partial trisomy of short arm of chromosome 4. Pregnancy was terminated. It was a male fetus with microcephaly, flat face, depressed nose with wide nasal bridge, synophrys and prominent heels. Facial features resembled de Lange syndrome but extremity findings and other features of de Lange syndrome were not present.

While there are some similarities between these two syndromes, clinical differences are also present. Report of Fryns on two cases with partial trisomy 4p followed till 18 and 24 showed more clear differences of this syndrome than same aged cases with de Lange syndrome. We also conclude like Fryns that this locus does not have any relationship with de Lange syndrome.

P0362. Subtelomeric cryptic rearrangement: further case of deletion 4p16.3

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The propositus, the first child of unrelated parents, was referred to us because of delay of psychomotor development and growth. Maternal age at delivery was 35 years; paternal age 38. They had the second healthy daughter. The family and history was unremarkable. During the pregnancy UltraScan revealed IUGR. The baby was born at 37 weeks with Cesarean section, birth weight 1750 g (<5th centile), length 42 cm (<5th percentile) and head circumference 29.6 cm (<5th percentile), APGAR score 8 and 9 at the first and fifth minutes respectively. At 25 months-old age showed weight 5350 g (<5th centile), height 71.5 cm (<5th centile), head circumference (OFC) 42.6 cm (<5th centile), triangular facies, sparse hair, large forehead, up-slanting palpebral fissures, hypertelorism, hypoplastic bulbous nose with rounded tip, short and protruding ears, thin lips, mild retrognathia, short fingers and asymmetric legs, hypotonia.

Laboratory test (thyroid, immunoglobulines, celiac disease) were normal. Also metabolic analysis (aminoacidemia and aminoaciduria, urine organic acids) were not significant.

Karyotype standard showed normal female set: 46, XX

Abdomen ultrasound, ECG, EEG and cerebral MRI were normal.

The subtelomeric rearrangements by FISH analysis showed deletion of the band 4p16.3:

ish del(4)(p16.3) (LSI WHS-). Another studies are in progress to define breakpoints and molecular size of deletion. Deletion band 4p16.3 cause Wolf-Hirschhorn syndrome (WHS) and Pitt Rogers Danks syndrome. Our case is further contribution to correlation genotype-phenotype.

P0363. Modeling of human minisatellite MS32 somatic instability in mouse embryonal teratocarcinoma cell line F9

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In order to elucidate mechanisms of hypervariable human minisatellite MS32 somatic instability, we transfected F9 cells with construct pcDNA3.1/MS32 containing 45 repeated units (29b.p. per unit) of MS32. F9 cells were transfected with the help of ExGene500 and selected on resistant to genetin. Transfection by pcDNA3.1/CAT was the positive control. There were obtained only 2 MS32-positive clones due to different transfection approaches (various time of transfection, DNA/ExGene 500 ratio; by linear and ring vectors). The efficiency of transfection was about tenfold less when compared with the positive control. However cells losing resistance to genetin during selection contained MS32 as revealed by PCR. Thus inefficient transfection could be a consequence of abnormal expression of genetin resistance gene in transfected cells. It is necessary to note, that FISH has not revealed sequences homologous to MS32 in F9 cells. However, computer analysis has shown an occurrence of sites with different degree of homology to MS32 in mouse genome. Maximal homology was revealed in interstitial regions of chromosome 11 (67%, P 0,01) and telomeric regions of chromosome 15 (63%, P 0,01). The MS32 propensity to be unstable could lead to structural rearrangements during or after integration event. Therefore nonrandom integration of pcDNA3.1/MS32 into silent areas of mouse chromosomal DNA can not be excluded as a result of recombination of host and foreign DNA repetitive sequences in such areas. Further molecular and cytogenetic analysis of obtained clones will enable an understanding possible reasons and mechanisms of somatic instability of human DNA repetitive sequences.

P0364. Precise determination of chromosome material and breakpoints in the patient with partial trisomy of short arm of chromosome 8 by CGH

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We report a patient a 13-year-old girl with dysmorphic features: mental and growth retardation, muscular hypotonia, hirsutism, brachycephaly, antimongoloid slant of eyes, synophrys, long curly eyelashes, short nose with anteverted nostrils, prominent philtrum, thin upper lip, downturned angles of mouth, short neck, keeled chest, small hands and feet with short fingers, clinodactyly of fifth fingers. Cytogenetic analysis with GTG of proband revealed gain of chromosome material on terminal part of 9p. Chromosomal in situ suppression (CISS) with DNA probe from a specific microdissected chromosome 9 confirmed a presence of additional chromosome material on the short arm of one homologue of chromosomes 9. Comparative genomic hybridization (CGH) was applied for identification of additional chromosome material and determination of breakpoint region. CGH-analysis of proband DNA revealed gain of chromosome material of the region 8pter→p21.3. Thus, the karyotype of patient was described as 46,XX,der(9)t(8;9)(p21.3;pter). This is a first case of CGH application in clinical cytogenetics for determination of breakpoints region and additional chromosomal material in patient with partial trisomy in Russian Federation.

P0365. A cytogenetic analysis of chromosome complement of spermatozoa from patient 45,Xr(Y)/45,X injected into mouse oocytes

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Structural rearrangements of Y-chromosome are common in infertile men. Carriers of such anomaly have an increased risk of spontaneous abortion and newborns with genetic abnormalities. To assess frequency and type of chromosome aberrations in spermatozoa from patient 45,Xr(Y)/45,X with severe oligoastenoteratozoospermia heterologous ICSI fertilisation between mouse oocytes and human spermatozoa was applied. The spermatozoa from fresh or cryopreserved-thawed samples were used. Totaly 91 suitable metaphase plates of human sperm chromosomes were received. Ratio of X- to Y-bearing spermatozoa was not significantly different from the expected 1:1 (χ^2 test, $P>0.05$). Frequency of spermatozoa with abnormal chromosome complements was higher in patient 45,Xr(Y)/45,X compared to 108 spermatozoa with normal head from men with normal sperm parameters - control group (68,25% and 23,15%, respectively) (ϕ -Fisher test, $P>0.05$). Compared to control group in spermatozoa of patient 45,Xr(Y)/45,X there was increased frequency of diploidy (4,71% vs. 0%), hyperploidy of gonosomes (11,64% vs. 1,85%), hypoploidy (48,24% vs. 12,04%) and frequency of structural aberrations (29,41% vs. 6,48%). There was no any interchromosomal effect and frequency of hyperploidy of autosomes did not differ from the same one of the control group (5,88% and 5,55% respectively). Our data suggest that cytogenetic analysis of spermatozoa from the carrier of structural reorganisation of chromosome can provide more information about proportion of normal and abnormal spermatozoa that will be useful for genetic counselling.

P0366. Autistic phenotype associated with a 15q11-q13 maternal triplication

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Here we report on a 4 years old boy with mild developmental delay, autistic behaviour and a chromosome rearrangement involving the 15q proximal region. A series of 8 BACs was used to characterise this rearrangement at the molecular level. A triplication was found. The triplicated segment was larger than the typical PWS/AS deleted region. The proximal breakpoint is located within BAC RP11-810K23, immediately proximal to BP1. The distal breakpoint is localised between BAC RP11-382B18 and RP11-758N13, located on each side from BP5. The middle segment was inverted compared to the distal and proximal segments. Molecular analyses showed that the rearrangement was of maternal origin.

Maternally derived triplication of the imprinted chromosome 15q11-q13 region have been reported previously in association with autistic spectrum disorders, cognitive deficits and seizures at least in ten patients. Three paternally derived triplications were also reported with a milder phenotype. Our observation confirms that 15q proximal triplication are most often of maternal origin. This rearrangement shares with SMC(15) a breakpoint located more distally than PWS/AS deletion breakpoint. Presumably, a common mechanism is involved in all cases of 15q proximal triplication since the middle segment is inverted in all cases studied at the molecular level.

P0367. FISH studies with tiling path clones applied to two chromosome 7 rearrangements.

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We report two patients with abnormal phenotypes in which conventional cytogenetics had shown *de novo* chromosome 7 rearrangements. FISH studies using tiling path clones http://www.ensembl.org/Homo_sapiens/cytoview) were undertaken to further refine the nature and structure of the

abnormalities. The first patient, a two month old female, was initially referred with dysmorphic features, short palpebral fissures, overlapping fingers, a history of poor feeding and possible Williams or Greig syndrome. Chromosome analysis showed an apparently balanced pericentric inversion involving bands 7p13 and 7q11.23. FISH revealed that the 7q11.23 breakpoint had split the BAC RP11-575M4, and may possibly have disrupted the gene AUTS2. The short arm breakpoint is between the BACs RP11-21H20 and RP-815D20 in 7p12.3. Further studies are required to determine whether the breakpoint involving AUTS2 or the short arm breakpoint accounts for this patient's abnormal phenotype. The second patient, a seven year old male, presented with mild to moderate learning difficulties, arrested hydrocephalus, short stature and a small head. Conventional cytogenetics reported an insertion of 7q31>7q34 into 7p14 and also suggested that a small deletion had occurred at the 7q31 breakpoint. FISH studies confirmed and refined the size and position of the 7q31 deletion and revealed a second cryptic deletion ~1.5Mb distal to the revised breakpoint at 7q35. These deletions or the breakpoints therefore may account for this patient's abnormal phenotype and studies are ongoing to determine the number and nature of the genes involved.

P0368. A familial translocation associated with complex partial epilepsy with a breakpoint within the Miller-Dieker syndrome deletion region on 17p13.3

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Miller-Dieker lissencephaly syndrome (MDS) is a microdeletion syndrome with facial dysmorphism, microcephaly, mental defect, seizures, spastic diplegia/spastic gait, decerebrate posturing and lissencephaly (smooth brain) with thickened cortex caused by a cortical migration defect. The 17p13.3 deletions associated with the MDS phenotype consistently include 8 known genes, from ABR at the telomeric end to SERPINF1 at the centromeric end. This region includes the LIS1 gene, point mutations or intragenic deletions of which are associated with isolated lissencephaly. Other anomalies of MDS appear to be the consequence of deletion of additional genes in this region. We have identified a mother and daughter with complex partial epilepsy, both of whom carries a balanced reciprocal translocation t(2;17)(q12.1;p13.3). By FISH mapping we localized the 2q breakpoint within the BAC clone RP11-98m17, a region without any known genes. In contrast, the 17p13.3 breakpoint mapped within the MDS region just distal to the 14-3-3-epsilon gene (YWHAE). Patients with deletions including CRK and YWHAE have more severe lissencephaly, suggesting that deletion of one or both of these genes may augment the lissencephaly phenotype (Cardoso et al. 2003). Our finding support that YWHAE or nearby genes may also be involved in the epilepsy in the present family, and thus, with the epilepsy frequently seen in MDS.

P0369. A chromosome 10 inversion, inv(10)(q11.22;q21.1), associated with mental retardation and autism

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Neuropsychiatric disorders in childhood including mental retardation and autism may be caused by genetic factors. Different strategies can be undertaken for the identification of these genetic factors. One approach is based on a detailed mapping of chromosomal rearrangements associated with specific phenotypes. Candidate genes may subsequently be identified from breakpoint regions. We have identified four unrelated individuals with a paracentric inversion on 10q by conventional high resolution karyotyping. Patient I has attention deficit hyperactivity disorder. Patient II has a mild mental retardation, autistic features and a congenital heart defect. Patient III has speech impairments, mild mental retardation and enlarged hands

and feet. Patient IV has a mild mental retardation. Fluorescence *in situ* hybridization (FISH) with chromosome 10 derived BACs in patient I and II confined the chromosome 10 inversion breakpoints to q11.22 and q21.1. The inverted segment is approximately 10 Mb. We suggest that the phenotype in these individuals is caused by the inversion. We hypothesize that the normal function for one or several genes in, or adjacent to, the inverted region is altered. Further experiments are in progress for the characterization of the breakpoints and, to identify candidate genes.

P0370. Submicroscopic 1p distal duplication, and monosomy for the short arm of chromosome 18 due to a paternal apparently reciprocal, balanced, translocation

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We report the study of a patient who carries an unbalanced segregant from a paternal, reciprocal, balanced translocation involving the whole short arm of chromosome 18, and a submicroscopic region at 1p36.3 band. A five-month-old female was referred for a genetic evaluation, due to pre- and post-natal failure to thrive, global mild psychomotor delay and multiple dysmorphisms.

Preliminary cytogenetic analysis showed, in the proband, an apparent "pure" whole 18p deletion.

Analysis of the parental chromosomes showed the father to carry an apparently balanced t(1;18)(p36.3;18p11.2). FISH with several probes, and MLPA technique allowed to identify the reciprocal translocation, with submicroscopic distal 1p duplication, and the whole 18p deletion, in the proband. The phenotype/karyotype correlation is the result of the overlapping of both 1 and 8 chromosomes rearrangements. Chromosome 1p duplications are rare: in the literature, few cases were reported of isolated 1p duplication, most of which were proximal or interstitial duplications, while the distal 1p36.3 duplication is exceptional. This study demonstrates the utility of subtelomeric specific FISH probes and MLPA methods, for detecting cryptic subtelomeric rearrangements in subjects with unusual clinical phenotype, without rearrangements at the routine cytogenetic studies, or with a clinical spectrum non-fitting with the identified chromosome abnormality.

P0371. Understanding infertility and missed abortion genetically: Experience on Indian population

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Chromosomal analysis was conducted on 2000 couples having infertility, recurrent pregnancy loss, repeated stillbirth or neonatal expiry, as an ultimate option, since the reason was unknown after investigation of almost all relevant parameters. Classical abnormalities were detected by conventional banding techniques as the sole reason in 6% of the cases in their genome. Variant abnormalities were much higher, over 20%. The abnormalities, including balanced translocation, inversion, deletion, duplication, etc. were observed transmitted to next generation with multiple congenital defects leading to still birth or neonatal death. Chromosomal study in abortus gives some causal information about repeated failure of reproductive outcome. In many instances, abnormalities present in men are causing primary infertility. Moreover, transmission of such constitutional aberrations results into very severe clinical expression due to recombination and leads to early onset of malignancy. These aberrations have no cure. Therefore, a chromosome study would be much more cost-effective, not only for the parents but also for the whole family and future generations to come.

P0372. Three unbalanced offspring from translocation carrier family

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We describe a familial reciprocal translocation between chromosomes 4p and 10q. Two family members (a father and one daughter) carried a balanced t(4;10)(p15.1; q 26.3) and three family members (two daughters and son) inherited a partial monosomy 10q 26.3 →pter and partial trisomy 4p 15.1 →pter and showed an unbalanced karyotype der(10) t (4;10)(p15.1; q26.3).

The first pregnancy in the family ended at 28 weeks of gestation with still-born child with malformations. Two sisters from the second and the third pregnancy are moderate retarded with dysmorphic features, speech delay, short stature. The sister from fourth pregnancy is phenotypically healthy. The boy from fifth pregnancy is severely mentally retarded without speech, short stature, dysmorphic features, cryptorchism.

P0373. Mosaic karyotype 45,X/46,X,idic(Y)(qter→p11 ::p11→qter) found in an ambiguous genitalia and short stature carrier.

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The carrier, a 11.6 years old child was referred for cytogenetic investigation because of her ambiguous genitalia (a small phallus with hypertrophy of labia majora), short stature for her age, and abnormal body proportion (short extremities, big muscles).

At birth, she had normal weight and length, but obvious genital ambiguity. She suffered a clitoroplasty at the age of 3, and received Naposim at age of 7- 8 years.

Karyotype was established in peripheral blood lymphocytes by GTG, and CBG-banding. There were found two cell lines: first, with 45 chromosomes in 90% of cells, and the second one, with 46 chromosomes in a frequency of 10%. GTG-banded karyotypes revealed monosomy X in hypodiploid cells, and a isodic(Y)(qter→p11::p11→qter) in pseudodiploid cells. The Y origin of isodicentric was confirmed by CBG-banding and FISH using SRY probe.

Both parents, revealed normal karyotypes in their peripheral blood. This strongly suggests that the error occurred during gametogenesis before spermatid stage, or during the first division after fertilization. Furthermore, the low percentage of cells carrying the idic(Y) may be due to the fact that, both centromeres are functional. The breakpoint on both chromatids seems to be subtelomeric on p arm, because Y euchromatin including SRY was present and active, judging her ambiguous genitalia.

The intraabdominal dysgenetic testis on the right part, and a fibrous structure on the left one was removed surgically. She had a very small uterus.

Monolayer tissue cultures were established from the surgically removed testis, and cytogenetic investigations are ongoing.

P0374. Uncommon variants of Turner syndrome detected before puberty

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Two ten years old girls were referred for cytogenetic examination because of their features

of Turner syndrome. One of them had also an additional autistic disorder with moderate mental retardation.

Chromosome studies on peripheral blood lymphocyte cultures, by conventional banding techniques, showed that they differ of classical Turner syndrome.

In the first case, after G- and C- banding, we find a mosaic karyotype 45,X/46,XY with chromosome Y detected in 50% of cells. C-staining method revealed a large C- band size

on two third of Yq arm, without ectopic C- heterochromatin in any autosomes. It is known that, only a proportion of subjects mosaic for Y material exhibit features of Turner syndrome, even in the presence of SRY gene. In the near future, our case must be investigated both for the presence of SRY gene, and for the evolution of her external genitalia.

The second case exhibits a 45,iXq karyotype. A structural abnormality such as iso Xq is quite common, but the additional autistic disorder is unusual in Turner girls. However, different chromosomal disorders account for less than 5% of all cases of autism, which supports the hypothesis that the autistic disorder may develop as a consequence of a chromosomal abnormality.

P0375. Two patients of terminal 6q25 deletion

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Terminal deletions at 6q25 are rare and about 30 cases have been reported. We report two additional patients with 6q25-qter deletions. Patient 1 was admitted to hospital because of status epilepticus at the age of 4 months. Brain MRI revealed hydrocephalus due to cerebral aqueductal stenosis. At 3 years of age he was moderately mentally retarded. His facial features were distinct with epicanthic folds, strabismus, nose with broad tip, long philtrum, thin upper lip, high and narrow palate, down-turned corners of the mouth, and large ears. Additional anomalies included microcephaly, fetal finger pads, hypermobility of the ankle joints, hypotonia, epilepsy and abnormal EEG. The chromosomes (400 bands) detected a terminal deletion of chromosome 6 at q25. Parental karyotypes were normal.

Patient 2 was floppy as a newborn and cranial ultrasound revealed large lateral ventricles. At the age of 18 months she needed hospitalisation because of seizures. At the age of 20 years she was moderately mentally retarded and the parents reported personality problems such as aggressiveness, stubborn temperament and tendency to withdraw. The face appeared long and slightly asymmetric. She also had epicanthus, strabismus, abnormal retinal pigmentation, nose with broad tip, down-turned corners of the mouth, high and narrow palate, and large ears. Additional anomalies included microcephaly, pectus excavatum, a deep sacral dimple, tapering fingers, bilateral hallux valgus, epilepsy and abnormal EEG. Chromosome studies (>550 bands) and FISH techniques detected terminal 6q25.3-qter deletion. Parental karyotypes were normal.

This report supports the existence of a terminal 6q deletion syndrome.

P0376. Rare proximal interstitial deletion of chromosome 4q in a girl with severe mental and growth retardation.

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Proximal interstitial deletions in the long arm of chromosome 4 have been reported in about 20 patients. Even though deletions show variable extensions spanning from 4q12 to 4q27, they all share the region q21-q22 causing similar phenotypes which include: short stature, cranio-facial abnormalities, limb, skeletal and cardiac anomalies, seizures, hypotonia, developmental delay and mental retardation. Here, we report a new case of a girl carrying a small 4q deletion including the 4q21 band. The abnormal chromosome 4 was characterised by G-banding and molecular cytogenetic methods including comparative genomic hybridisation and two-colour fluorescent in situ hybridisation with band-specific probes. The girl presented with short stature, severe mental retardation, normal head circumference, brachydactyly, frontal bossing, deep-set eyes, anteverted nostrils, thin upper lip, corpus callosum hypoplasia, anomalies of the heart inter-ventricular septum and fasting hypoglycaemia. Even though her deletion was one of the smallest described so far, she shared more than 15 typical signs of the 4q21-q22 deletion syndrome with other patients carrying larger deletions. It is conceivable that genes in the 4q21 band be responsible for most of the features of the 4q21-q22 deletion syndrome.

P0377. X;Y translocation associated with dyschondrosteosis

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We report a case of a 24-years old female with dyschondrosteosis. The basic cytogenetic examination (G-banding) found an abnormal karyotype with derivative chromosome X [46, X der(X)] resulting from X;Y translocation. The C-banding of the abnormal chromosome indicated presence of Yq heterochromatine region on the terminal part. Translocation breakpoints were located on p-arms of both chromosomes.

FISH analysis was carried out with locus specific (Kallman, SRY and

XY-subtelomeric loci), X-centromeric and Y-heterochromatine probes. It confirmed the cytogenetic result and determined possible breakpoints on chromosomes Xp22 and Yp11. Xp- and Yp-subtelomeric (DXYS129) and SRY regions were not identified on the derivative chromosome. Our results indicate that the translocation is associated with the deletion of XpYp-pseudoautosomal and SRY regions. This loss of chromosomal segments could explain the phenotype of the patient. We suppose that dyschondrosteosis probably resulted from haploinsufficiency of SHOX homeobox gene on the XY pseudoautosomal region. These data confirm conclusions of previous reports.

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P0378. Combined cytogenetic and molecular evaluation of infertile men

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Genetic factors are one of common cause male infertility. To evaluate the frequencies of genetic abnormalities in different sperm diagnoses we examined cohort of 605 Russian infertile men. Sperm analysis that performed according WHO recommendations (1999) revealed: azoospermia in 36%, oligoasthenoteratozoospermia (OAT) in 35%, asthenozoospermia in 14%, teratozoospermia in 7%, normozoospermia in 8% patients. Cytogenetic analysis was carried out on blood lymphocyte metaphases by standard method with G-staining. Detection of Y-chromosome microdeletions was performed according to Laboratory guideline Simoni et al. (1999). Genetic abnormalities were found in 44 (20%) azoospermic men: in 26 (59%) - Kleinfelter syndrome, in 4 (9%) - structure chromosome aberrations, in 5 (11%) - XX-males (de la Chapelle syndrome), in 9 (21%) - AZF deletions. In patients with OAT genetic abnormalities were revealed in 8% cases (17 men). At this structure chromosome aberrations (8 cases) are most common (47%). Kleinfelter syndrome was mentioned in 4 men (24%), one XX-male (6%), Y-microdeletions were found in four men (23%). In asthenozoospermic men genetic abnormalities disclosed in 5 cases (6%): structure chromosome aberrations - 4 (11%), one patient with karyotype 47,XYY. Of 40 teratozoospermic men in 2 (5%) cases were revealed structure chromosome aberrations. Between normozoospermic patients one men (0.5%) had balanced chromosome translocation 46,XY,t(Y;14)(q12;p12).

P0379. Satellite association and Ag-NOR staining in lymphocytes from patients with Fanconi Anemia

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Satellite associations (SA) and silver stained nucleolus organizing regions (Ag+NORs) of acrocentric chromosomes in metaphases obtained from lymphocytes of patients with Fanconi Anemia (FA) were studied. Peripheral blood samples from seven patients with FA, six patients with anemia other than FA (non FA) and six normal healthy individuals (controls) were used in this study. Phytohemagglutinin (PHA) stimulated peripheral blood cultures were performed, and satellite associations and Ag-NOR staining were determined at day 3 of culture. Our results showed that the frequency of cells with satellite associations, and the number of acrocentric chromosomes involved in association per cell were significantly decreased in patients with FA compared with controls and non FA. Moreover, a significant lower percentage of Ag-NOR positive acrocentric chromosomes was seen in FA patients. No significant differences were observed between non FA and controls, both in the frequency of cells with satellite associations and the number of acrocentric chromosomes involved in association per cell. This results may suggest that FA patients have a significant decrease in nucleolar organizer activity and that this cytogenetic pattern distinguishes FA patients from patients with other types of anemia, which may be considered an additional feature to be used in the cytogenetic diagnosis of FA patients.

P0380. Molecular characterization of an inversion duplication of the long arm of chromosome 4

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Here we report on a 7 years old boy, first child of healthy non consanguineous parents, with psychomotor developmental delay, dysmorphic features and neurobehavioral problems. Facial dysmorphism is characterized by a long face, macrocephaly, a broad nasal bridge, narrow palpebral fissures, a very little mouth and little squared ears. The hands are short. Behaviour is characterized by an extreme aggressiveness. Clinical follow up showed sinus bradycardia and persistence of behavioural problems. Cytogenetic analysis showed an abnormal long chromosome 4q. Parental karyotypes were normal. To characterize this *de novo* chromosome rearrangement at the molecular level, FISH studies were performed. The subtelomeric probe RP11-173M11 located on 4q35.2 was deleted whereas probe RP11-294M24 and RP11-90E13, located respectively on 4q34 and 4q33 showed an inverted duplication of this region. A series of 15 BACs were used to characterize the size of the terminal deletion (9.2 Mb, from the BAC clone RP11-783P19 located on 4q34.3 to 4qter) and the extent of the inverted duplicated segment (11Mb, from the BAC clones RP11-243F3 located on 4q33 to RP11-783P19 located on 4q34.3). The mechanism of this rearrangement and the behavioural disorders are discussed

P0381. Peculiarities of pericentromeric heterochromatin replication pattern of chromosomes 1, 9, 16 in embryonic and extraembryonic tissues of human fetuses

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The investigation of DNA replication is an awarding approach for analysis of chromosome functional organization. Replication time (S) is strongly correlated with chromatin transcriptional activity. Therefore the analysis of replication pattern of heterochromatin regions in metaphase chromosomes from embryonic and extraembryonic tissues at different stages of embryonic development allows indirect estimation of heterochromatin role in differentiation of embryonic cells and chorionic villi. The technique of immunofluorescent detection of BrdU with monoclonal anti-BrdU antibody was applied for analysis of replication pattern in metaphase chromosomes of chorionic villi cells as well as of tissues fragments from 8 human embryos at 5 and 13 weeks of gestation. The number of G-bands, replicating simultaneously with heterochromatin regions of 1q12, 9q12 and 16q11.2 in cytotrophoblast cells gradually decrease in all samples from 5 to 13 week. A high level of asynchronous replication (22% - 55%) of homologous regions (1q12, 9q12, 16q11.2) in both embryonic and extraembryonic tissues was revealed. Time changes in the replication pattern of relevant heterochromatin regions most probably reflect some unknown peculiarities of their functional activities in embryonic and extraembryonic tissues at different stages of embryonic development.

P0382. Gene regulation in coronary atherosclerotic plaques from patients with stable angina pectoris and acute coronary syndromes via nuclear remodeling

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Introduction. Thrombus formation over a ruptured atherosclerotic plaque in a coronary artery is the key event leading to myocardial infarction and unstable angina. Tissue factor (F3) has been found more abundant in coronary atherosclerotic plaques from patients with ACS with respect to those experiencing SA thus suggesting that its content may determine different thrombotic response to plaque rupture

in human coronary arteries. We aimed evaluating whether there is a phenotypic commitment of tissue factor expressing cells within the atheroma and whether a nuclear involvement of chromosome domains occurs.

Material and Methods. Different cell types were grown out from explanted ACS and SA plaques. Total mRNA was extracted and Real Time PCR was performed to assess the expression pattern of Tissue Factor (Chromosome 1p22-p21) and its inhibitor WISP3 (Chromosome 6q22-q23). *In situ* RT-PCR followed by FISH analysis with chromosome painting were performed on fixed cells to asses the relationship between the F3 expression and chromosomal positioning within the nucleus.

Results. We found that vascular smooth muscle cells (VSMCs) were the major source of intra-atheroma F3. Moreover, tissue factor from ACS VSMCs was 68 times more expressed than in SA plaques ($p < 0.01$). FISH analysis revealed two distinct mutually exclusive arm domains for both Chromosome 1 and 6 being the two clustered together in ACS and apart in the SA plaques. The expression of F3 was enhanced when the two chromosomes were closer to the nuclear periphery. Thus we demonstrated a cell-specific orchestration of gene activity in SA and ACS via nuclear remodelling.

P0383. Balanced translocation breakpoint mapping with BAC-FISH in patients with normal phenotypes.

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Imbalances or disruptions of genes at chromosome breakpoints have been found in recent studies to be the underlying cause of the association in some patients between a clinically abnormal phenotype and an apparently balanced *de novo* reciprocal translocation (Gribble et al J Med Genet 42:8-16 2005). Our hypothesis that translocation breakpoints in normal individuals would neither disrupt genes nor have imbalances was tested by analysing the breakpoints in 13 phenotypically normal individuals incidentally ascertained with familial balanced reciprocal translocations. The breakpoints were refined by fluorescence *in situ* hybridization (FISH) using tiling path BACs to identify breakpoint-spanning clones. Array CGH (1 Mb) was also performed to identify imbalances not directly involved in the breakpoints. No breakpoint associated imbalances were identified in any of the 13 cases, but in the majority, Ensembl mapping data suggested that the breakpoints might disrupt a gene at one or both breakpoints. In two cases, breakpoints which clearly disrupt pathogenic genes were identified, i.e. the genes were physically larger than the BACs which were split by the breakpoints. By array CGH, one of the patients had a *de novo* deletion in a chromosome not involved in the structural rearrangement. Further detailed FISH studies showed one translocation to be non-reciprocal. This study confirms that patients with a normal phenotype and apparently balanced reciprocal translocations do not have FISH detectable breakpoint-associated imbalances, but may have genes disrupted and possibly inactivated by a breakpoint(s).

P0384. De novo rearrangements in Wolf-Hirschhorn syndrome: characterization, parental origin and parental 4p16.3 inversion polymorphism.

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Wolf-Hirschhorn syndrome (WHS) is a contiguous gene syndrome caused by partial 4p deletion, with genomic rearrangements representing a *de novo* event in most cases.

We analysed a total of 59 WHS patients. Genetic analyses included: a) conventional and molecular cytogenetics (4p-specific probes and all telomeres); c) microsatellite segregation analysis; d) multiple colour FISH for the 4p16.3 inverted polymorphism in parents. We observed that: a) the basic genomic rearrangements were *de novo* events in 54 out of 59 patients (91.5%); b) they were isolated deletions in 43/54 patients (80%); unbalanced translocations in 7 (13%), consisting of

t(4p;8p) (5 cases), t(4p;11p) (one case), t(4p;7p) (one case); dup/del in 3 (5.5%); double intrachromosomal rearrangement in one (1.5%).

A total of 27 families were simultaneously tested for type of rearrangement, parental origin and parental 4p16.3 inversion polymorphism. We found that:

1) Rearrangements were paternal in origin in 22 out of 27 cases (80%). Nearly the totality of them were isolated deletions (21/22), with the only exception of a dup/del rearrangement. The 4p16.3 inversion polymorphism was absent in both parents in each case.

2) Rearrangements were maternal in origin in the remaining 5 cases (20%). They all were unbalanced translocations: t(4p;8p) (4 cases) and t(4p;7p) (one case).

A maternal 4p16.3 inverted polymorphism was detected in 3 out of 4 WHS associated t(4p;8p) translocations, it was absent in one case of t(4p;8p) and in the case of t(4p;7p).

Relevant considerations are discussed.

P0385. Sperm chromosomal aneuploidies in 421 severely oligozoospermic men with and without constitutional genetic alterations who are candidates for intracytoplasmic sperm injection

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Concerns have been raised regarding the potential of intracytoplasmic sperm injection (ICSI) to facilitate the transmission of genetic diseases. Sperm of severely infertile men might carry genetic alterations either because a constitutional abnormality is present or because only sperm are altered as a result of mitotic or meiotic errors due to impaired spermatogenesis. We studied 421 severely oligozoospermic men (sperm count below 5 mil/mL) for sperm sex chromosome aneuploidies by means of FISH. They included: 7 patients with complete (47,XXY) and 3 with mosaic (47,XXY/46,XY) Klinefelter's syndrome, 4 with reciprocal or Robertsonian translocations, 11 with Yq microdeletions, and 9 with CFTR mutations. Patients with complete Klinefelter's syndrome had a significantly lower percentage of normal Y-bearing sperm and an higher percentage of XX- and XY-disomies (6.1 \pm 1.0% and 13.3 \pm 2.2% respectively, p<0.001). Patients with Y chromosome microdeletions had a significantly lower percentage of normal Y-bearing spermatozoa, and an increase in nullisomic sperm and XY-disomy (10.4 \pm 1.5% and 4.1 \pm 1.2%, respectively, p<0.001). Subjects with CFTR gene mutations showed no alterations, confirming that spermatogenesis proceeds regularly in these patients. No significant difference was found in men with mosaic Klinefelter's syndrome or translocations due to the low number of subjects. The analysis in idiopathic patients revealed an increased percentage of genetically abnormal sperm with a significant increase in XX-, YY-, and XY-disomies (0.6 \pm 0.8%, 0.6 \pm 0.8%, and 1.4 \pm 1.8, respectively, p<0.001). Our findings may explain the higher prevalence of congenital anomalies in children conceived by ICSI and in particular in children conceived to oligozoospermic men.

P0386. Localization of chromosomes 13, 16, 18, 21, 22, X and Y in nuclei of human preimplantation embryos

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The aim of the study was to find a possible correlation between chromosomal constitution of blastomeres from preimplantation embryos and nuclear localization of chromosomes with regard to the known peripheral localization of chromosome 18 and inactive X in female somatic cells. Screening for aneuploidy of chromosomes 13, 16, 18, 21, 22, X and Y was performed in 98 blastomeres from human embryos in 22 IVF cycles. Vysis Multivision PB and CEP X + CEP Y Alpha probes were used. Relative distances from the center and the edge of nucleus of 1173 FISH signals were exactly measured in digitized microscope images. We compared localization of signals of all analyzed chromosomes from euploid and aneuploid (all types of aneuploidy of studied chromosomes) blastomeres by homogeneity tests in contingency tables. The actual signal localizations were compared with a theoretical model describing a random distribution

of signals in the 3-dimensional nucleus by chi-square goodness-of-fit test. Localization of chromosome 18 in all (euploid + aneuploid) cells was significantly (P=0.001) different from the theoretical model with shift to the periphery. A significant (P=0.007) shift to periphery was also found for chromosome 18 in aneuploid compared to euploid blastomeres. Such shift was not found for other chromosomes including 18 in euploid and X in XX blastomeres. These findings indicate that aneuploidy might alter gene activity of other chromosomes by the shift to nuclear periphery. We have not found X inactivation accompanied by peripheral localization of X chromosome in XX blastomeres.

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P0387. Quantitative fluorescence PCR examination of parental and meiotic origin of trisomy 21 in Ukraine, Russia and Central Europe

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We have studied parental and meiotic origin of free trisomy 21 in 102 nuclear families from different regions of Ukraine, Russia and Central Europe using quantitative fluorescence PCR on ABI Prism 310 by 8 STR markers. The aim of the study was to find out possible impact of different lifestyle, load of exogenous noxious agents including radiation from Chernobyl accident. The proportion of maternal non-disjunction was 77.3% in Germany, 93.8% in Ukraine and 92.3% in Russia, whereas the paternal origin was disclosed in 13.6%, 6.2% and 7.7% respectively. In different regions of Eastern Europe the prevalence of meiosis I errors was 84.4% of maternal origin, 3.1% of paternal in Ukraine and 77.1% of maternal origin, 2.9% of paternal origin in Russia. The disorders in meiosis II occurred in 9.4% or 14.3% of maternal and 3.1% or 5.7 of paternal non-disjunction in Ukraine and Russia respectively.

Our findings are consistent with published data and the differences are not statistically significant as followed from homogeneity test in contingency tables and chi-square test.

If these observations are confirmed by further analyses of 150 Russian and Ukraine nuclear families with trisomy 21, it would suggest, that different life style, noxious exogenous factors load and Chernobyl radiation exposure have no impact on proportions of parental and meiotic origin of trisomy 21. It might be due to the negative selection of trisomic embryos in the early phase of their embryonal development.

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P0388. Variant translocations and the forms of BCR/abl rearrangement in chronic myeloid leukemia

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The Philadelphia (Ph) chromosome arises from a reciprocal translocation, with *c-abl* oncogene from 9q34 fused to the breakpoint cluster region (BCR) locus on chromosome 22q11. In 5-10% of patients with CML, Ph chromosome originates from other rearrangements different from classical t(9;22)(q34;q11). A simple Ph-producing translocation involves chromosome 22 and other chromosomes than 9, while a complex variant translocation involves three or more chromosomes. In almost all cases with "variant" Ph chromosome, the BCR/abl rearrangement can be detected at the molecular level or by *in situ* hybridization.

In this study, variant translocation in 19 patients with newly diagnosed

CML, was presented. Cytogenetic analysis was performed on bone marrow cells after direct preparation, according to modified method of HG-banding. Two (2/19) patients had simple variant Ph-producing translocations, and other (17/19) patients had complex variant translocations.

Reverse transcription-polymerase chain reaction (RT-PCR) for detection of the expression of *BCR/abl* sequence in eight CML patients was performed. *BCR/abl* rearrangement was found to be expressed in b3a2 form in six (6/8) patients, but in b2a2 form in one (1/8) patient. Using RT-PCR analysis in one (1/8) CML patient with simple variant translocation, we found that *BCR/abl* rearrangement was not expressed.

Results of cytogenetic and molecular investigations in cases of "variant" Ph chromosome CML reviewed in this study clearly indicate that variant translocations of chromosomes 9 and 22 are great research challenge, especially in case of rearrangement forms of *BCR/abl* hybrid gen that can be different from each other and different from specific rearrangements (b2a2, b3a2) for CML.

P0389. Cytogenetic Abnormalities in the Lymphocytes of Iranian Breast Cancer Patients

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Breast cancer is the most common malignancy among women. Several factors are involved in initiation and development of breast carcinoma, but epidemiologic evidence point to two areas: Environment and Genetics. Cytogenetic studies in breast have revealed chromosomal alterations in tumor cells. Most breast tumor cytogenetics studies thus far reveal complex chromosomal alterations, and hence it has not been possible yet to associate breast cancer with specific chromosome aberrations, although some specific anomalies are beginning to emerge. Blood cultures from breast cancer patients also have revealed chromosomal changes which are similar to those of tumor cells. Such similarities may be very interesting, since it indicates that factors causing alterations in tumor cells may also operate in human lymphocytes. In the present study we have observed such similarities.

P0390. Cytogenetic Analysis of Reproduction Problems

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We investigated 501 married couples with reproductive disorders of different origins. Cytogenetic diagnostics was performed on preparations of metaphase and prometaphase chromosomes (G and C banding). We analyzed 24 thousand of metaphase plates and 2 thousand of prometaphase plates.

These results were systemized according to the etiology of reproductive losses. Abnormalities and chromosome variants occur in all groups of men and women with primary infertility. It was detected that women with primary infertility have more high frequency of chromosome abnormalities than women with secondary infertility and spontaneous abortions of different etiology ($7,2 \pm 2,5\%$ against $2,2 \pm 0,9\%$, $p < 0,05$). It was found that 23,8 % of men has variants of chromosome in group of primary infertility, and only 7,1 % - women ($p < 0,001$). Men with the primary infertility have more higher inversion-frequency of heterochromatic region of chromosome 9 than women of the same group (8,7 % against 1,6 %, $p < 0,05$). Chromosome variants of karyotype were found among women with spontaneous miscarriages and primary infertility in anamnesis and equal $20,0 \pm 4,0\%$, $7,1 \pm 2,3\%$ correspondingly ($p < 0,05$). Among men and women with secondary infertility and spontaneous miscarriages of different genesis 29,4 % of karyotype disorders were met within men and 19,7 % - women. The results have been used for choosing infertility treatment tactics.

P0391. Accuracy of array-based CGH in genome-wide detection of submicroscopic chromosome aberrations: comparison with conventional CGH

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Genome imbalances are a major cause of developmental delay and congenital anomalies. Cytogenetic techniques for the study of genomic diseases include karyotyping and comparative genomic hybridization (CGH). Novel high resolution whole-genome technologies such as array-based comparative genomic hybridization (array-CGH) have recently been developed. Array-CGH significantly improves the detection rate of submicroscopic chromosomal abnormalities. Using a tiling resolution array consisting of 14.000 BAC clones covering the entire human genome, we studied 15 patients with chromosomal abnormalities including unbalanced translocations, deletions and duplications. All patients have previously been analysed by other cytogenetic techniques like conventional CGH and/or FISH. Overall, good concordance was shown between array-based CGH and conventional CGH. The regions of copy number change were correctly identified by array-CGH in a one-step experiment. The higher resolution of array-CGH allows us to more precisely define the size of the deletion or duplication. In addition tiling resolution arrays enable us to assign breakpoints within single BAC clones. This method is therefore becoming a powerful tool for the rapid and accurate detection of genetic disorders associated with genomic copy number abnormalities. Array-CGH in combination with conventional cytogenetics will significantly improve clinical genetic diagnosis.

P0392. Detection of submicroscopic chromosomal imbalances in patients with learning disability and dysmorphic features by array-based comparative genomic hybridization (array-CGH)

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The underlying causes of learning disability and dysmorphic features in many patients remain unidentified despite extensive investigation. Routine karyotype analysis is not sensitive enough to detect subtle chromosome rearrangements (less than 5 Mb). We previously investigated the presence of subtle DNA copy number changes in patients with learning disability and dysmorphism by array-CGH, employing a DNA microarray constructed from large insert clones spaced at approximately 1 Mb intervals across the genome (Shaw-Smith et al, J Med Genet, (2004) 41:241-8). We have now studied a total of 100 patients and identified 23 copy number changes in 22 patients (one patient had two large imbalances on different autosomes). All of these imbalances have occurred in distinct genomic regions; none of them was detected by routine karyotype analysis. Nine of the copy number changes were de novo occurrences, whereas five were shown to have been inherited from phenotypically normal parents. Studies are in progress to determine the origin of the remaining nine imbalances. Five of the imbalances involved a single clone from the 1 Mb array; all of the remainder involved two or more consecutive clones. One patient with the phenotype of atrioventricular septal defect and behavioural difficulties was found to have a de novo deletion at 8p23.1. This deletion narrows the critical region for behavioural difficulties at chromosome 8p23.1. On the basis of these results, we anticipate that array-CGH will become a routine method of genome-wide screening for unbalanced rearrangements in patients with learning disability.

P0393. Evolution of the cytogenetic techniques: follow up of a case through out 20 years

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In 1984 a couple was referred to our unit due to recurrent miscarriages. A cytogenetic study was performed and the karyotypes obtained were 46,XY and 46,XX, t(14;21)+mar. The female parents were then studied and it was found out that both the translocation and the marker chromosome were inherited from the father and previously

from the grandparent. Since the FISH technique was not available it was supposed that the marker chromosome was derivative from the robertsonian translocation.

In 1985 the woman got pregnant and underwent a prenatal diagnosis (PD) resulting in a carrier female of both the translocation and the marker chromosome. The follow up after birth revealed a normal development. Afterwards she had two consecutive miscarriages and in 1987 she underwent a new PD where the karyotype obtained was 47, XX, + mar. At birth it was noticed vesicoureteral reflux and mild microcephaly, so a new cytogenetic study was performed revealing a 47,XX,+mar/48,XX,+mar,+mar2 karyotype.

At eleven years old learning difficulties were observed, with no major developmental delay. The FISH analysis showed that the origin of the family marker was chromosome 15.

The result from FISH analysis of the secondary marker was D1Z7/D5Z2/D19Z3+ and D1Z5-. After a review of the literature we have decided to perform the analysis of chromosomes 5 and 19 with the QF-PCR in order to ascertain the parental origin of this chromosomes and discard a parental disomy. This study is actually being performed.

P0394. Molecular cytogenetic characterisation of terminal chromosome 2q37 deletion in patients recruited through the A.C.L.F. telomere network.

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Studies have ascertained associations between autism or Albright hereditary osteodystrophy-like phenotype and terminal 2q deletion with the breakpoint within 2q37.3. Furthermore subtelomeric 2q37.3 deletions are often detected with FISH subtelomeric screening and most of those 2qtel deletions are considered polymorphisms as they are also observed in the parents. However some of them are de novo terminal deletions associated with an abnormal phenotype. We collected 14 observations from 10 new families through the A.C.L.F. (French Speaking Cytogenetician Association) telomere network, in order to better characterise the different length of the 2q37 deletions observed. We analysed for each patient and his parents when available, the size of the deletion by molecular cytogenetic and molecular biology with STS markers. The size of the deleted segment was determined by mapping the region with specific BAC and PAC clones from 2q36 breakpoint to the subtelomeric 2q end, and with different polymorphic markers from D2S125 to D2S2585.

The children were aged from 18 months to 14 years. They were all investigated for developmental delay. They had all facial dysmorphic features but looked different from each other. At least 4 of them presented multiple malformations including, cardiac malformation, coloboma, syndactyly, brachydactyly, cryptorchidism, ano-rectal malformation, or cleft palate. None of them were referred for Albright osteodystrophy-like phenotype, and one had autistic behaviour.

We present the clinical, chromosomal and molecular cytogenetic and genetic findings of the affected children and their parents investigated, comparing our results with the literature data. (Study supported by the CHU-Reims-AOL 2003).

P0395. Trisomy 15 mosaicism associated with a familial reciprocal translocation t(1;15).

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We describe a 4 years old female with severe global mental retardation (< 1st centile, Griffiths scale), myoclonic epilepsy, proximal hypotonia, microcephaly (<3rd centile), kyphosis, cutaneous syndactyly between 2nd and 3rd finger of left foot, hands stereotypy and minor facial dysmorphisms.

A second trimester amniocentesis, performed elsewhere, had disclosed a reciprocal translocation 46, XX; t(1;15)(q12;p11)mat. The balanced reciprocal translocation was found in the healthy mother, in a healthy

aunt and in grandmother, but also in the 5-years-old cousin, who showed overlapping phenotypical features (severe mental retardation, epilepsy, microcephaly, hypotonia and minor facial dysmorphisms).

We performed the constitutional karyotype at high resolution, adding WCP1 and 15, FISH screening for subtelomeric rearrangements, STS analysis to exclude UPD for chromosome 15 and an extensive work up for mosaicism. The results allowed identifying a 2% trisomy 15 mosaicism in peripheral blood: mos47,XX, t(1;15)(q12;p11)mat +15/46,XX; t(1;15)(q12;p11)mat. The cytogenetic polymorphisms of the supernumerary chromosomes 15 exclude with confidence an in vitro mitotic error.

Unfortunately, the proband's cousin was suddenly dead.

Fryns reported a 2-3% additive risk for congenital abnormalities and mental retardation in the heterozygous children of healthy translocation carriers. This risk may be overestimated, however many mechanisms can be put forward to explain an increased risk: upd, position effect, cryptic unbalanced defect and postzygotic loss of a supernumerary chromosome, with only partial rescuing from a tertiary trisomy resulting in trisomy mosaicism. We discuss this latter, in particular for translocations with high probability of 1:3 segregation, as expected from pachytene configuration.

P0396. De novo mosaic 46, XX, dup (11)(q13q25)/46,XX in a patient with trigonocephaly

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The most common form of dup(11q) is associated with 11q/22q translocation, and in almost all reported cases, it is inherited through a carrier parent. Here we report on a 2-year old female with trigonocephaly and multiple congenital anomalies. She was born to first cousin parents with unremarkable family history. Pregnancy ended at term with normal birth weight. She was noted to have microcephaly, trigonocephaly, upslanting palpebral fissures, prominent nasal root, thin upper lip, and micrognathia. Echocardiogram revealed VSD and PDA. She also had horseshoe kidneys, congenital dysplasia of the hips, and moderate developmental delay. Blood chromosome analysis revealed two cell lines. Based on 50 cells examined, duplication 11q (q13q25) was observed in 24% of cells and normal 46,XX in the remaining cells. Parents chromosome analysis was normal.

This case presents several unusual findings. It is a de novo chromosomal aberration while the vast majority of reported cases with dup(11q) inherited the anomaly through a carrier parent. The mosaicism of this particular karyotyping is another extraordinary finding. The duplicated region (q13-q25) includes the more commonly reported duplication seen in the unbalanced segregants of t(11;22) carrier. Finally, it is the first reported case of trigonocephaly in association with 11q duplication, in contrary to the well-known association of this specific type of craniosynostosis with the 11q23 deletion syndrome. However, FISH analysis using an MLL (11q23) DNA probe showed only two signals with loss of signal of the proximal MLL gene on the duplicated chromosome while it was present at the distal 11q23.

P0397. Molecular study of miscarriages with unbalanced reciprocal translocations.

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Spontaneous miscarriages occur in approximately 10-15% of clinically recognised pregnancies. Chromosomal anomalies account for at least 50% of first trimester miscarriages. Carriers of balanced translocations have an increased risk of having abnormal or unviable products of conception. Conventional karyotyping of abortions presents a high rate of failure. In contrast, molecular studies of DNA (QF-PCR and CGH) provide a useful tool to diagnose numerical chromosome anomalies. We present the molecular approach of two abortions derived from couples in which one partner carried a balanced reciprocal translocation. Case 1 shows complete trisomy 2 due to a 3:1 segregation from a paternal t(2;17)(q32.1; q24.3). Case 2 shows partial monosomy 13q32 > qter and partial trisomy 1q31.2 > qter caused by an adjacent-I segregation of a maternal t(1;13)(q31.2; q32). In both cases, parental karyotypes and DNA were available. Trivalent segregation could be established by analysing STR markers. We propose QF-PCR in addition to CGH as an

efficient diagnostic method to improve our knowledge of unbalanced offspring in balanced translocation carriers.

P0398. Direct duplication 12p11.21-p13.31 mediated by segmental duplications: a new genomic disorder?

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We have identified an almost 20 Mb direct duplication (dual-colour FISH analysis) of the short arm of chromosome 12 in a child with mental retardation and dysmorphic features.

The molecular definition of the breakpoints, performed by array-CGH and FISH analysis, showed that they were within two highly homologous segmental duplications of 49 and 123 kb respectively. Thus this rearrangement is a typical genomic disorder.

Pip-maker analysis demonstrated three clusters of homologous segmental duplications located at 3, 9 and 31 Mb respectively from the 12p telomere, constituted by portions with both direct and opposite orientation. Microsatellites analysis demonstrated the paternal origin of the rearrangement. Paternal chromosomes 12 have been inspected for the presence of inversion with the same breakpoint of the duplication, predisposing to the duplication itself but resulted normal. An analysis of the literature to see if this duplication was recurrent revealed several cases of 12p duplication that, at least at the cytogenetic level, were bigger than this one. Similarly, reciprocal deletions are not reported in the literature likely reflecting their lethality.

This case demonstrates that not only recurrent rearrangements such as deletions involving 7q11.2, 15q11q13, 22q11.2 but also sporadic rearrangements may be mediated by low copy repeats.

P0399. Phenotypic variability associated with an unbalanced X/Y translocation transmitted through females in a three generation family

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We present the phenotypic variability of an unbalanced X/Y translocation, in a three generation family - grandmother, mother and a two year old male child (propositus). The grandmother has a normal phenotype but short stature, the mother has short stature and mild mental retardation. The propositus has a more severe phenotype with short stature, developmental delay, cryptorchidism and dry skin compatible with a contiguous gene-deletion syndrome.

Conventional and molecular cytogenetics (FISH) revealed the presence of a derivative X chromosome, shared by the three family members: der(X)t(X;Y)(p22.3;q11.2).ish(WCPY+, DXYS29X-, EsTCdy16c07++, DYZ3-, SRY-).

In order to identify the extension of the Y chromosome segment, and the Xp22.3 deletion on the der(X), PCR with specific primers for Yq11.2 and Xp22.3 was performed. Y chromosome sequences present belong to Y intervals 5 and 6, with the breakpoint occurring between sY89 (absent) and sY98 (present). The deletion on the Xp22.3 covers all the genes from the pseudoautosomal X region 1 (PARX1) to the X specific segment until DDX237 marker. The breakpoint in Xp22.3 is between DDX237 (absent) and DDX278 (present), showing that the disease causing genes, SHOX, ARSE, VCX-A, and STS are absent. Most of the propositus clinical features can be explained by the Xp23 partial nulism. The propositus doesn't exhibit ichthyosis and we speculate that this could be explained by the activation of an alternative pathway that overcomes the X-linked steroid sulfatase deficiency. The phenotypical variability in the two female patients, namely the degree of cognitive impairment, could be related to a non-random X chromosome inactivation.

P0400. Non-contiguous 8p23 deletions in a case with del(8)(p23.1)/psudic(8)(p23.2) mosaicism

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Mosaicism with two cell-lines carrying different abnormalities for the same chromosome are quite rare among constitutional chromosome anomalies. In these cases it can be assumed that the zygote would have had a 46 chromosome karyotype but one of the chromosomes was dicentric (for example: qter->p::p->qter or pter->q::q->pter). The presence of two active centromeres on the same chromosome could lead to the formation of anaphase bridge during cell division followed by an asymmetric breakage of the dicentric with the formation of a deleted chromosome and an inv dup del chromosome.

We report a double 8p anomaly mosaicism del(8)(p23.1)/psudic(8)(p23.2) in a girl with severe mental retardation, dwarfisms and corpus callosum agenesis. The dicentric breakpoint does not span any of the dupicons associated with the classical inv dup(8p) (Giglio et al, 2001).

The rearrangement is of paternal origin and the proposita's father has the 8p23.1 heterozygous inversion between the two olfactory-receptor gene clusters (REPD and REPP), located respectively at 7 and 12 Mb. The deleted 8p chromosome has two non-contiguous deletions involving the distal 7 Mb and a 3 Mb region located between 9 and 12 Mb from the telomere. These results suggest that the presence of the cryptic paracentric inversion in the paternal 8p chromosome, from which originated the proposita's dicentric chromosome, gave rise to two non-contiguous deletions in an unique breakage event.

These data underline the importance of a proper molecular analysis to characterize chromosome anomalies in order to an accurate karyotype-phenotype correlation.

P0401. Characterization of a de novo 20q13 subtelomeric deletion by microarray comparative genomic hybridization in a child with learning difficulties

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Unbalanced subtelomeric chromosomal rearrangements represent a significant cause of unexplained mental retardation with or without congenital abnormalities. Development of new molecular cytogenetic techniques has provided a powerful tool for detection of patients with subtle chromosome imbalances.

We report the case of a 5-year-old girl with learning difficulties, hypotonia and strabismus. Delivery occurred by C-section at 34 weeks of gestation because of preeclampsia. Birth measurements were above the 90th percentile, but term was uncertain. Facial phenotype was not dysmorphic. Standard karyotype (resolution 550 bands) was 46,XX. Subtelomeric FISH analysis revealed a submicroscopic *de novo* deletion of 20q13. To further characterize the size of the deletion and in order to delineate the critical region involved in the phenotype of the patient, we performed comparative genomic hybridization on DNA microarrays, a technique which allows simultaneous detection and mapping of DNA copy number changes. We used commercially genomic microarray slide (Human Array, Genosystem, France) containing 480 non overlapping BAC/PAC clones spotted in triplicate. By CGH-microarray the deletion was confirmed by three PACs deleted and its size is estimated to be between 0,4 Mb and 2.1 Mb. Despite the extent of this microdeletion 20q13, there doesn't seem to be a clear pattern of phenotypic expression associated with this chromosome anomaly.

P0402. Are chorionic villi a source of stem cell?

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The aim of this study was to investigate for the presence of stem cell in chorionic villous sampling (CVS), from women subjected to prenatal diagnosis at X-XII week of pregnancy and to the develop an in vitro culture system for their maintenance and expansion.

Three CVS were analyzed at the time of sampling for Alkaline phosphatase (AP) positivity, while pan-cytokeratin and vimentin were

used as markers for epithelial and mesenchimal derived cells. In freshly collected CVS few clustered AP positive cells were present in the apex of some villi.

To better characterize AP positive cells and differentiating cells from CVS, we performed primary cell culture up to obtain cell monolayers after 15 days of culture.

We observed that, after 15 days of culture, AP positive cells were present and expanded exclusively in cells cultured in the presence of 1000 UI of Leukemia inhibitory factor (LIF). At this time most of the cultured cells were positive for vimentin.

Experiments to analyse these AP+ cells for stem cells markers as Oct-4, Nanog and SSEA 1-4 are in progress, both by molecular and immunohistochemical studies.

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P0403. Success of intracytoplasmic sperm injection in infertile men with chromosome abnormalities

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Purpose: To assess the success rates of ICSI according to the type of male chromosomal abnormalities.

Methods: We analyzed infertile men with chromosomal abnormalities undergoing ICSI: seven men from the Slovene population and 87 men from 37 published articles.

Results: Altogether 94 men with chromosomal abnormalities underwent ICSI. In couples with male nonmosaic Klinefelter syndrome 36 children with normal karyotype were born and one 47,XXY fetus was conceived. In 19 couples with male reciprocal translocation eight children were born: four with normal karyotype, three with balanced translocation and one with unbalanced translocation. In 28 couples with male Robertsonian translocation 14 children were born: six with normal karyotype and eight with Robertsonian translocation

Conclusions: ICSI is an efficient method for patients with chromosome abnormalities, however prenatal diagnosis or preimplantation diagnosis should be offered.

P0404. Mosaic cell line in a dysmorphic child with sacrococcygeal teratoma

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Marker chromosomes represent a heterogeneous group of chromosomes which have different expression in phenotype of an affected person. Some of them are silent, and could be transmitted through generations, but some could give a distinct phenotype, depending of the length and the origin of an extra chromosome. Mosaicism, if present, affects different tissues in different percentage. Teratomas, on the other hand, have heterogeneous etiology. Sometimes they represent a part of a syndrome (Curranino syndrome, caused by mutations in HLXB9 gene), but several reports show various cytogenetic findings in these children -partial trisomy of 13q, 20p, 1q, 19p, 2p; most often as a result of an unbalanced translocation complement. Marker chromosomes are rarely seen as an etiology of sacrococcygeal teratomas.

We report a newborn with a sacrococcygeal teratoma, heart defect and facial dysmorphism. It was a first child of young and unrelated parents. The pregnancy was uneventful; the baby was born premature, with birth weight and length under 3rd percentile. The baby had dysmorphic signs including small face, deep set, small eyes, frontal bossing, saddle nose, hypoplastic alae nasi, short philtrum, V-shaped mouth, and micrognathia. Also the baby presented heart defect, diastasis m. recti abdominis, brachydactyly, transversal crease, hypoplastic nails, digitalized thumb. Sacrococcygeal teratoma was present, surgery confirmed connection of the tumor with the rectum, with a histology of a mature teratoma. Karyogram of the leukocytes showed two cell lines 46,XX/47,XX+mar;(80%/20%). Extra material on this marker chromosome predisposed altered embryogenesis and development of teratoma in the child.

P0405. Characterization of two Silver-Russell syndrome patients with uniparental disomy of chromosome 7

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Silver-Russell syndrome is characterized by intrauterine and postnatal growth retardation, asymmetry of the head and limbs, a small triangular face and other less constant features. A subset of patients (7-10 %) has been described to show maternal uniparental disomy of chromosome 7. We have characterized a total of 12 Silver-Russell patients and found 2 of them to be positive for chromosome 7 uniparental disomy. The first case was found to be caused by maternal isodisomy except the terminal region of the long arm, that was heterodisomic. In the second case we found the patient to show maternal isodisomy of the whole chromosome. In addition, we observed that in the second patient, the chromosome 7 microsatellites besides manifesting maternal isodisomy, had also a second very faint band corresponding to one of the paternal alleles. Cytogenetic analysis of both patients, their parents and prenatal amniotic fluid could not detect any chromosomal abnormalities or mosaicism.

P0406. Clinical - cytogenetic aspects related to mosaicism in the variants of Turner syndrome

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Turner Syndrome distinguishes itself among the abnormalities of the sexual chromosomes by a large variety of karyotypes and phenotypes. Most of the Turner patients are mosaics. We have investigated 90 patients with feminine morphotype. The growing retard was the most frequent symptom of the Turnerian stigmata, associated with absent or hardly present secondary sexual characters, primary or secondary amenorrhea. For all the observed subjects a genetic consult was performed, followed by a chromosome analyses from lymphocyte cultures. The karyotype was established after the G banding procedure. For each case, there were examined 50 metaphases. Our goal was the cytogenetic diagnosis of the Turner Syndrome, the emphasizing of its cytogenetic variants, and the correlation karyotype-phenotype.

The non-mosaic karyotypes included cases of 45,X, 46,X,1(Xq), 46,XX,del(Xq), and 46,XXdel Xq-; Xp-, 46,Xr(X) and a normal karyotype 46, XX. Ten mosaicism cases were registered with a cellular line 45,X or 46,XX. Those cases consisted of: 2 cases of numeric abnormalities 45,X/46,XX, the rest being structural abnormalities: 45,X/46,X(r)X [2]; 46,XXdel(X)(q22.3-22.8) (p22.1 - pter)/45,X; 46,XXdel Xq-; Xp-/45,X; 46,XX/46,XXdelXq-[2]; 45,X/46,Xi((Xq) [2].

The deletions of the regions Xq2.1 and 1.3 were associated with a primary amenorrhea and the terminal deletions Xq , with a secondary amenorrhea, early installed. The phenotypic aspect of the patients with structural X abnormalities varies according to the chromosomal abnormality and not to the clones' percent in the mosaic conditions. Most of the mosaics with 45,X may be the consequence of a mitotic instability of the chromosomes with a structural abnormality.

P0407. Homozygosity for pericentric inversions of chromosome 9 in a parent with stillbirth

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An inversion occurs when a single chromosome undergoes two breaks and is reconstituted with the segment between the breaks inverted, and are of two types: paracentric and pericentric. An inversion does not usually cause an abnormal phenotype in carriers, because it is a balanced rearrangement. Its medical significance is for the progeny; a carrier of either type of inversion is at risk of producing abnormal gametes that may lead to unbalanced offspring. One of the most frequent inversions is inversion chromosome 9. A few cytogeneticists consider inversion of chromosome 9 as a normal variant. However, many reports in the recent literatures link pericentric inversion of chromosome 9 with infertility, recurrent abortions and a number of

other abnormal conditions.

Up to now just a few cases of homozygosity of inversion of chromosome 9 have been reported. We report a case of homozygosity pericentric inversions of chromosome 9 in a woman with 28-week stillbirth. Our cytogenetic studies on her both non-consanguineous parents showed that both parents were heterozygote for this inversion with a normal phenotypes.

P0408. The high incidence of mosaic forms of numerical chromosome abnormalities in spontaneous abortions detected by interphase multiplex FISH

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The leading cause of spontaneous pregnancy loss in the first trimester are numerical chromosomal abnormalities. Interphase multiplex fluorescence *in situ* hybridization (MFISH) is considered as effective technique for accurate determination of numerical chromosome abnormalities in uncultured cells. MFISH using original DNA probes for chromosomes 1, 9, 13, 14, 16, 18, 21, 22, X, Y was applied for investigation of aneuploidy and polyploidy in 259 spontaneous abortions (mean age of gestation: 9.8 weeks). The mean maternal age was 30.3. The frequency of abortions with abnormal karyotypes was 54.8 % (142 specimens). Among them 8 specimens (5.6%) were characterized by multiple chromosome abnormalities. The occurrence of autosomal aneuploidy was 48.5% (65 from 134), X chromosome aneuploidy – 25.4% (34 from 134), and polyploidy – 26.1% (35 from 134). The high incidence of specimens with chromosomal mosaicism was detected. Among 142 specimens with chromosome abnormality 64 (45.1%) have mosaic forms of aneuploidy and polyploidy. We conclude MFISH to have high efficiency for screening of regular as well as mosaic forms of numerical chromosome abnormalities in spontaneous abortions. Supported by INTAS grant 03-51-4060.

P0409. The gain and loss of chromosomes and somatic chromosome pairing in developing human brain.

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Molecular cytogenetic studies supply the pilot evidences for increased level of aneuploidy in developing and adult human brain (Yurov et al., 2001;2005). However the real frequency of aneuploidy in the developing brain cells is still unknown. FISH artifacts due to over position of interphase chromosomes and somatic chromosome pairing can lead to "pseudomonosomy" registered by interphase FISH. Therefore, the resolution power of FISH analysis of somatic (diploid) cells is limited. The application of qualitative and quantitative assessment of single signals in interphase nuclei of fetal brain (as described in Iourova et al. 2005) allowed us to establish the incidence monosomy and of somatic chromosome pairing. In the present study we have applied interphase FISH with DNA probes for chromosomes 1, 9, 15, 16, 18, X, and Y for identification of aneuploidy with quantitative assessment of FISH signals in 6 fetal human brain samples. No fewer than 3000 interphase nuclei was scored per probe for each sample. We have detected non-specific aneuploidy incidence of all the chromosome studied. The variation of aneuploidy frequency was in the range 1.3-2.4% for monosomy and 0.3-0.4% for trisomy and rare tetrasomy. The somatic chromosome pairing or over position of FISH signals was detected to take place for all the chromosomes studied and varied in the range 2.9-7.3%. Therefore low-level chromosomal mosaicism as well as somatic chromosome pairing do present in fetal human brain. Supported by INTAS grant 03-51-4060.

P0410. FISH Analysis of Subtelomeric Chromosome Rearrangements Detected in 108 Malformed Infants and/or with psychomotor delay.

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Chromosome alterations are usually associated to infants with malformations and/or psychomotor delay. Sometimes it has normal karyotype. We observed that high resolution G-band karyotype (550-850 bands) can hide cryptic chromosome alteration not detected but with fluorescence "in situ" hybridization techniques (FISH). Some cryptic chromosome alterations affect subtelomeric region.

We started a project aimed to analyze subtelomeric regions in about 300 infants with congenital defects and/or psychomotor delay with normal high resolution (550-850 bands) G-band karyotype (PI020028). We analyzed 108 infants, all with normal high resolution G-band karyotype. We have established two groups: A: 42 newborn with congenital malformations with/or without psychomotor delay. B: 66 dysmorphic children aged 1 to 13 years, with psychomotor delay. We used the subtelomeric FISH probes from Cytocell and Vysis.

Among the 108 cases, 10 had a subtelomeric rearrangement [9.26% (4.44-17.03)]. Three rearrangements were found in group A [7.14% (1.47-20.87)] and 7 rearrangements in group B [10.61% (4.26-21.85)].

In 8 cases the chromosome alteration was "de novo" [1p deletion (2 cases), 4p deletion (3 cases), 10p deletion (1 case), 10q deletion (1 case) and a der(18)t(18;19)(1 case)]. In two cases the father was carrier of a balanced chromosome translocation (Table 1).

Our results show the importance to perform subtelomeric chromosome rearrangements with FISH in newborn malformed infants and malformed infants with psychomotor delay, even they have a normal high resolution G-band karyotype (550-850 bands), since this could help defining the prognosis of the patient and to give a correct genetic counselling to the family.

Table 1.- Chromosome alterations detected after FISH with subtelomeric chromosome probes.

1°	Newborn	del(1)(p36.22) de novo"
2°	newborn	der(22)t(12;22)(q24.31;q13.3)pat
3°	newborn	del (4)(p16.3). ish 4p16.3 (WHSCRx2) de novo"
4°	No newborn	del(4)(p16.3). ish 4p16.3 (WHSCR-) de novo"
5°	No newborn	del (4)(p16.3). ish 4p16.3 (WHSCRx2) de novo"
6°	No newborn	der(4)t(4;12)(q34;p12.3)pat
7°	No newborn	del(10)(q26.3) de novo"
8°	No newborn	del(1)(p36.22). de novo"
9°	No newborn	der(18)t(18;19)"de novo"
10°	No newborn	del(10)(p15.3) de novo"

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P0411. Chromosomal transcriptome maps and linkage to sequence data

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The enormous potential enabled by current techniques to study genome wide gene expression profiles, opened new avenues in biological/medical research. Especially in cancer research, insights into the expression profile can provide vital diagnostic and prognostic information. Current micro-array techniques are extremely powerful and may yield direct information on the gene activity of a cell or tissue. However, special equipment and a sophisticated bioinformatics program are required to evaluate data and no direct geographical expression profile on the chromosome is provided. The CESH (comparative expressed sequence hybridization) technique on the other hand represents a unique technique to directly assign loci of high/low gene expression to specific chromosomal loci (Lu et al. 2001). As normal human metaphase spreads are used as target to visualize hybridized cDNAs, we searched for a way to directly link expression- with sequence data. Until today, no direct link from the sequence information to the chromosomal phenotypes exists. Thus, we attempt to find a way to directly link the GC-content published in accessible data banks like Ensembl and information obtained by staining chromosomes with base specific fluorochromes by following the concept of linking clearly defined landmarks on the chromosome (high or low fluorescence intensity with base specific fluorochromes like chromomycin A3 (CMA) with GC-rich or GC-pure domains in the genomic sequence. So far we were able to demonstrate that the GGCC binding motif correlates well with the (CMA) staining. This correlation allows e.g. a direct link of the expression profile along the chromosomes as obtained by the CESH technique with the sequence information accessible by different data banks. Thus, the use of sequence specific fluorochromes allows a precise assignment of chromosomal bands to the genomic sequence. So not only the ISCN information will be available but also every chromosomal position can be described in Mbp units.

P0412. The role of M-FISH in simultaneous detection of *de novo* marker chromosomes

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INTRODUCTION: The identification of marker chromosomes and the elucidation of their clinical significance remains one of the few problems in classical human cytogenetics. This will undoubtedly yield to the application of M-FISH, which allows the simultaneous characterization of all human chromosomes. Because of the variety of marker chromosomes, their different origins and the possibility of imprinting and isodisomic effects, there is a great variation in the clinical outcome. Thus, the characterization of prenatally detected marker chromosomes identified in prenatal diagnosis is of great interest for future medical care.

MATERIALS AND METHODS: Ten cases involving *de novo* marker chromosomes were studied. All of the above cases were initially studied with the standard RGH chromosomal banding technique followed by complementary C-banding and NOR-staining techniques. M-FISH karyotype was performed using the commercially available kit of Metasystems GmbH. The labelled metaphases were visualized through a microscope equipped with appropriate filters and ISIS software (Metasystems GmbH).

RESULTS: In all studied cases we were able to successfully identify all marker chromosomes, even the smallest in size. The chromosomal constitutions were the following: +der(10)[1], +der(12)[2], +der(15)[1], +der(16)[3], +der(18)[1] and +der(22)[2].

DISCUSSION: The correct identification of all ten *de novo* marker chromosomes, in our prenatal cases, validates M-FISH as an invaluable tool for a high degree of accuracy and efficiency. Whereas there are several studies of fragments derived from sex chromosomes, the clinical significance of markers derived from regions of autosomal chromosomes is less clear. Unfortunately, the outcome for some of

these pregnancies was not possible to be traced, thus being unable to evaluate the phenotypic and clinical consequences of each case. Although M-FISH technique is expensive and not widely performed, its systematic application could well offer the information needed to form more precise evaluations regarding the management and counselling in *de novo* marker chromosomes found prenatally.

P0413. The contribution of apoptosis and aneuploidy assessment in sperm after repeated ICSI failure

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The analysis of DNA Fragmentation Index (DFI) by apoptosis using *in situ* fluorescent TUNEL technique and the assessment of chromosomal nondisjunction by FISH techniques on germ cells, provide valuable information concerning the paternal contribution on fertilization rate, early embryo abnormalities and IVF failures. In order to establish the DFI and the nondisjunction rate, 22 patients with a minimum of two ICSI attempt failures and/or less than 40% of fertilization rate were studied with a total of 29 ejaculates. For apoptosis the kit by Roche was used with the application of positive and negative controls. For nondisjunction assessment, FISH technique was applied for chromosomes 13, 18, 21, X and Y. A minimum of 2000 sperm nuclei were examined by two observers for each analysis. The data of our study indicates a variable DFI from one patient to another and from one ejaculate to another concerning the same patient. In 4 patients the DFI was less than 10%, in 7 ranged from 10 to 40%, in 10 was over 40% while 1 case was inconclusive. Concerning the rate of chromosome nondisjunction and DFI, no clear relation was established although 3 patients presented more than 70% of apoptosis, with more than 2% of multiple chromosome nondisjunction.

P0414. Array CGH reveals unexpected genomic imbalances in fetuses with multiple malformations and normal karyotype

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Malformations are a major cause of morbidity and mortality in full-term infants. Genomic imbalances are a significant component of their aetiology. However, up to one-half of patients with multiple congenital malformations remains unexplained despite thorough clinical examination and laboratory investigations. In the present study, we used a commercially available array CGH, on which all subtelomeric regions, main microdeletion syndromes, and 201 other probes covering the genome, on 49 fetuses selected with three or more significant anomalies and normal karyotype to detect submicroscopic chromosomal imbalances. Array CGH identified 8 genomic rearrangements (16.3%), all confirmed by Quantitative Multiplex PCR of Short fluorescent Fragments method. Subtelomeric and interstitial deletions, submicroscopic duplications, and a complex genomic imbalance were identified. In 4 *de novo* cases (15qtel deletion, 16q23.1q23.3 deletion, 22q11.2 deletion, and mosaicism for a rearranged chromosome 18), the genomic imbalance identified was clearly underlying the pathological phenotype. In one case, the relationship between the genotype and phenotype was unclear, since a subtelomeric 6q deletion was detected in a mother and her 2 fetuses bearing multiple malformations. In 3 cases, a subtelomeric 10q duplication, that is likely a genomic polymorphism, was identified. The detection of 5/49 causative chromosomal imbalances (or 4/49 if the 6qtel deletion is not considered as causative) argues for the relevance of a wide genome screening when the standard chromosome analysis is normal and confirms that array CGH will have a major impact on pre and postnatal diagnosis, as well as providing information for more accurate genetic counselling.

P0415. Non-invasive fetal RHD and RHCE genotyping from maternal plasma in alloimmunized pregnancies

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In this prospective study, we assessed the feasibility of fetal RH genotyping by analysis of DNA extracted from maternal plasma samples of alloimmunized pregnant women using real-time PCR and primers and probes targeted toward RHD (exon 7 and exon 10) and RHCE (exon 2 and exon 5) genes.

We analysed anti-D, antiD+C and anti-E alloimmunized pregnant woman at risk of haemolytic disease of the newborn (HDN) at first and second trimester of pregnancy and correlated the results with serological analysis of cord blood.

Determination of fetal D allele of RHD gene and the C and/or E alleles of RHCE gene from maternal plasma samples is highly accurate and enables implementation into clinical diagnostic algorithm for following pregnancies at risk for HDN. The detection of the negative fetus in the current pregnancy excludes the risk of HDN caused by alloantibodies and performance of invasive fetal-blood sampling.

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P0416. A New Class of Chromosomes Lacking Centromere Discovered During Prenatal Diagnosis and Characterized via Chromosome Microdissection and FISH

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The karyotypes of approximately 0.05% of the human population exhibit a small extra chromosome in addition to the normal 46 chromosomes. As might be expected, the majority of the marker chromosomes have been shown to contain centromeric material, e.g. alpha-satellite DNA (α-satellite DNA). However, a rare class of marker chromosomes that lack detectable α-satellite DNA has been reported. In the present study, we have employed chromosome microdissection technology to characterize a marker chromosome with no detectable α-satellite DNA which otherwise could not be identified by standard banding techniques or FISH.

Case Report: A small, mosaic, C-band negative marker chromosome was detected on culture of amniocytes during prenatal diagnosis related to advanced maternal age. Following spontaneous premature labor at 29 weeks gestation, a dysmorphic infant was delivered, with flat nasal bridge, short palpebral fissures, micrognathia, high forehead, low-set .

The origin of the marker chromosome was subsequently identified via chromosome microdissection. Through reverse FISH, we found the marker to be an inverted duplication of the region 15q26.1-qter. FISH with alphoid satellite probe was negative, while whole chromosome 15 paint was positive. Both ends of the marker chromosome were positive for the telomeric TTAGGG probe. These data, plus the G-banding pattern, identified this as an analphoid, inverted duplicated marker chromosome, lacking any conventional centromere.

We discuss the etiology and clinical effects of this marker chromosome, comparing it to the few other reported cases of "tetrasomy 15q" syndrome. We will also discuss the possible mechanisms that are likely responsible for this neocentromere formation.

P0417. Chromosomal anomalies in first trimester miscarriages

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It is well known that a large proportion of first trimester spontaneous abortions are caused by chromosome disorders. In the present study, chromosomal analysis of 259 consecutive first trimester miscarriages revealed an abnormal karyotype in approximately 61 % of the cases. Autosomal trisomies were most frequently detected (in 37 % of the

karyotyped samples), followed by polyploidies (9 %), and monosomy X (6 %). Cases with an extra sex chromosome constituted approximately 5 % of the karyotyped abortions, with a remarkably high frequency of 47,XXY (3.4 %). It is interesting to note that this is approximately 40 times greater than the prevalence of Klinefelter syndrome. Similar to the case of autosomal trisomies, our data suggests that the majority of conceptions harboring sex chromosome changes do not survive to term.

Autosomal trisomies, and an extra X-chromosome in males (47,XXY) were associated with an advanced maternal age, whereas monosomy X as well as polyploidy changes seems to be inversely related to the age of the mother.

The single most common aberration was trisomy 16, which was found in 14 % of the chromosomally abnormal abortions. We also identified one miscarriage with a full trisomy 1. This represents the third case of trisomy 1 in a clinically recognized pregnancy. Thus, all human chromosomes have been detected in the trisomic state in material from spontaneous abortions.

P0418. Pre-implantation Genetic Diagnosis (PGD) for Genetic Disorders in Saudi Arabia

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Saudi Arabian culture is highly consanguineous, with the first cousin marriages accounting for 60-70% of all marriages. Given the difficulties in management of genetic disorders, preventive measures for the suffering families from autosomal recessive disorders, by offering pre-implantation genetic diagnosis is undertaken. The first of these disorders is Sanjad-Sakati Syndrome (SSS) OMIM# 24140, which is characterized by congenital hypoparathyroidism, growth and mental retardation with a unique 12bp deletion. The second is Niemann Pick Disease type B (NPD-B) OMIM# 257200, (acid sphingomylinase (ASM) deficiency) with more than 70 mutations have been reported in (SMPD1) gene, which presents with severe phenotype in Saudi Arabia. Four unique mutations are found in our Saudi families. A family with (W533R) mutation in the (SPMD1) gene suffering from a severe phenotype underwent PGD. The third disorder is Morquio's disease (MPSIV) OMIM # 253000, with severe classic phenotype with N-acetyl galactosamine-6-sulfatase deficiency (MPSIV-A). More than 20 different mutations in (GALNS) gene have been reported in (MPSIV-A). A family with three affected siblings with severe classic (MPSIV-A) with detected W195C mutation in the (GALNS) gene underwent PGD. In all these three families PGD was undertaken using fluorescent PCR(F-PCR) and/or nested PCR with sequencing on a single cell. A singleton pregnancy ensure after transfer of one heterozygous and one normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia.

P0419. Preimplantation genetic diagnosis for spinocerebellar ataxia type 1

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Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder whose classical clinical features include ataxia, dysarthria and bulbar dysfunction. Death usually occurs between 10 and 15 years after the onset of symptoms. The disease is caused by expansion of CAG trinucleotide repeats within the coding region of the SCA1 gene on chromosome 6p22-23. Normal SCA1 alleles range from 6 to 39 CAG while clinical symptoms have been reported in individuals carrying 39 to 81 continuous CAG tracts.

We report for the first time a preimplantation genetic diagnosis (PGD) protocol based upon the co-amplification of the CAG repeats together with each of D6S89 and D6S260 microsatellites. These markers are closely linked to the SCA1 locus and have therefore potential diagnostic value through linkage analysis. A family requested PGD for SCA1 as i) the father displayed characteristic SCA1 features with an expanded allele of 46 CAG repeats and ii) their first pregnancy investigated by standard

prenatal diagnosis was selectively terminated as the fetus was carrier of the paternally transmitted expanded allele. Blood samples were collected from the couple and their unaffected daughter, then duplex single cell protocols for CAG/D6S89, and CAG/D6S260 have been tested on 119 and 121 single lymphocytes, respectively. PCR efficiency ranged from 94.9 to 100% and allele drop out (ADO) rates were comprised between 6.2 and 9.9%. The assay we set up is considered to be robust enough to proceed to clinical application, and therefore, a PGD will be performed for this family in the forthcoming weeks.

P0420. Psychological and ethical problems of prenatal diagnostics

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Prenatal diagnostics involving problems of fertility is a very sensitive field of medical care. Geneticists and other physicians are obliged to respect the feelings of future probable parents. Quite often the solution of problems is not ideal from the medical point of view, but it is the parents who must decide how to solve the situation. Our study illustrates some extreme situations of the families and their solutions.

Case 1: The wife 36 years old is six years older than her husband and she refuses prenatal diagnosis, because she is afraid of abortion and losing her husband.

Case 2: The husband is an adopted child, they would refuse abortion in any situation, they refuse amniocentesis.

Case 3: The pregnant women had two brothers with hydrocephalus. They both died 20 years ago, so it is not possible to do any DNA diagnosis. There is a possibility of X-recessive inheritance. They both refuse possible abortion of fetus 46, XY. They also refused prenatal diagnosis, including detailed USG examination.

Case 4: The pregnant women is 37 years old. By IVF she had one healthy son, who died at age two years of a brain tumor. Now she is pregnant (natural way) and she agrees with amniocentesis and they both are optimistic and brave.

We shall demonstrate 4 more cases.

Summary: The genetic counselling should be always emphatic, fully informative and non-directive.

P0421. Non-invasive prenatal diagnosis of fetal rhesus status by molecular analysis of maternal plasma

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Feto-maternal rhesus D (RhD) incompatibility is a serious and potentially life-threatening condition for the growing fetus. The correct determination of fetal RhD status is useful in the treatment of sensitised RhD-negative pregnant women. Traditionally, the fetal rhesus D status determination has been accomplished by invasive prenatal diagnosis (amniocentesis and chorionic villus sampling). Recently, the non-invasive prenatal diagnosis using fetal DNA isolated from maternal peripheral blood became available for antenatal determination of fetal RhD genotype.

The aim of the current study was to develop the non-invasive prenatal diagnosis method for fetal RhD typing using fetal DNA separated from maternal plasma.

Peripheral blood samples were obtained from 40 pregnant women at 9-40 weeks of gestation. DNA was extracted from maternal plasma and fetal RHD genotyping was performed in parallel using conventional PCR and real time PCR (Q-PCR).

Fetal RHD genotyping was performed for 18 RhD-negative and 22 RhD-positive pregnant women. Of 18 fetuses with RhD-negative mothers, 6 were identified as RhD-negative and 12 as RhD-positive. The results of PCR genotyping were compared with newborn's serology test to determine the sensitivity and specificity of the applied PCR methods. Two fetal samples were misdiagnosed as RhD-negative, while the postpartum serology screening revealed the RhD-positive blood types. The sensitivity and specificity of PCR typing of fetuses of rhesus negative pregnant women were 85,7% and 100%, respectively.

The results of the current study demonstrate that the non-invasive fetal RHD genotyping can be performed rapidly and reliably with the use of fetal DNA obtained from maternal plasma.

P0422. Cytogenetic studies of spouses who have experienced early miscarriage

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A miscarriage is often caused by chromosome aberrations. The structural errors in chromosomes found in the parents tested, most frequent of which are translocations, can be directly related to the development of chromosopathy in a fetus. However, there is still not enough evidence that minor structural variants may be a predisposition of a disturbed fetal genesis and an early miscarriage.

The objective of our study is to define correlation of minor chromosome structural aberrations in parents and chromosopathy in fetus by analyzing karyotype of a miscarried fetus and karyotype of parents. The analysis was carried out in 186 miscarried fetuses in the first trimester in 44 (23,6%) of which chromosome aberration was detected. Karyotype analysis was made in 44 spouses (88 examinees) of whom chromosopathy was confirmed in the fetal tissue. Chromosome structural aberration was found in 52 examinees (59,0%), two translocations, one inversion, one X chromosome mosaicism and 48 minor structural variants. Among the minor structural aberrations a satellite DNA was noticeable in chromosomes of D and G group, associations of chromosomes, very big or small hetero- chromatin segment of Y chromosome long arm.

We have suggested the possible cause-effect relation of heterochromatin polymorphism in parents and chromosome aberration in the fetus of such parents.

Key words: chromosome aberration, miscarriage, karyotype

P0423. No HLXB9 mutation in a human sirenomelia associated with tracheoesophageal fistula

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Sirenomelia is characterized by fusion of the lower extremities. Concordant monozygotic twins has been reported (Roberge, 1966), as recurrence in a family (Rudd, 1990), and a strain of mice, *srn* (Orr, 1982) Here, we report the first genetic study of sirenomelia.

One young non-consanguineous couple were referred because of sirenomelia, bilateral renal agenesis and sacral spina bifida at 11 weeks ultrasonography. Terminaison of pregnancy (TOP) was done at 14 weeks. Fetal karyotype was 46,XY. Pathology shows a single lower limb, without external genitals, nor anal orifice. Epidermized sacral spina bifida, bilateral kidneys agenesis, and absence of bladder was confirmed. A tracheoesophageal atresia was discovered. There was a single umbilical artery. X-rays showed a complete disorganization of lumbo-sacral vertebrae, with hemivertebrae in D7.

Association of sirenomelia and VATER signs has been described (Duncan et al., 1993; Harika et al., 1995). The association of sirenomelia with tracheoesophageal atresia is frequent (Stocker JT and Heifetz SA, 1987). Pathogenesis is controversial, precocious blood vascular steal was suggested. Another hypothesis is the anomaly of major gene of the formation of mesoderm, explaining both the vertebral defects and tracheoesophageal anomalies. Search for mutations in HLXB9 gene, responsible for the Currarino triad, was first tested, without found mutations. Extension of the study will be done, on brachyury gene and receptor of retinoic acid, CYP26A1. An international collaborative study is proposed.

P0424. Prenatal diagnosis of mosaicism identified in amniotic fluid cell cultures

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Chromosomal mosaicism is one of the most difficult problems to be solved in prenatal diagnosis. Mosaicism involving normal and abnormal karyotypes detected in cultured amniocytes may be a true chromosomal mosaicism or pseudomosaicism. Distinguishing between

true or pseudomosaicism usually needs a second prenatal diagnostic test to detail the genetic counselling.

In this presentation, we report two numerical and two structural mosaisms detected in amniocyte cultures. The first fetus had a 47,XY,+mar[10]/46,XY[10] karyotype. The marker chromosome was shown to be derived from chromosome 15 by FISH method. The delivery was by caeserean section. The newborn had intrauterine growth retardation and cerebral thrombosis and died at 29th day of age. The second fetus had a 45, X[4]/ 46,XX[26] karyotype. The parents refused cordocentesis and decided to terminate pregnancy in the 23rd week.

Third case had a 46,XX, dir dup(1)(q22-q32)[9]/46,XX[21] karyotype. The parents' karyotypes were normal and the pregnancy was aborted in the 23rd week of gestation. Second structural abnormality was reported as 46,XX,t(6;11)(q23; p13)[3]/46,XX[20]. The mosaicism was detected in only one flask. The parents decided to continue pregnancy and cordocentesis could not be performed due to the fetal and placental position. Fetal USG revealed no abnormalities. The gestation is at 32 weeks at the time of abstract submission.

All cases had different chromosomal abnormalities. Information and risks were explained to all families during genetic counselling. Mosaicism in prenatal diagnosis needs both detailed examination and follow up, since clinical findings depend on the type of abnormality.

P0425. Single Cell Quantitative Fluorescent Multiplex PCR in Preimplantation Genetic Diagnostics

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In this study we are reporting about developing and optimizing the protocol for quantitative fluorescent multiplex PCR (QF-PCR) based preimplantation genetic diagnostics (PGD) for *in vitro* fertilization (IVF). A great majority of patients seeking IVF are women >35 years old, who are at a higher risk of having children with trisomies 13, 18 and 21. Our assay includes a number of specific highly polymorphic STR markers for 13, 18 and 21 chromosomes (D13S634, D13S742, D13S258, D18S391, D18S 386, D18S941, D21S11, D21S1411 and IFNAR) and sex chromosomes (AMG). The main problem of single cell PCR is a dramatically low quantity of a genome DNA, but our PCR conditions allow us to solve it. After a cell lysis and a single round of QF-PCR products were detected on an automated DNA analyzer ABI 310 by using a Gene Scan technology. The two-round PCR also was developed, which will allow us to identify most common chromosome aneuploidies as well as point mutations leading to monogenic disorders. All our results were confirmed by routine cytogenetic methods. We have previously successfully tested this system on prenatal samples with 95 % efficiency and ~99,9 % accuracy. The efficiency of this method for single cells (blastomeres and lymphocytes) is 80 % and accuracy is near 95 %. Our optimized protocol for multiplex QF-PCR based PGD is an effective and accurate instrument for a preimplantation sex and trisomies 13, 18 and 21 detection, allowing rapid prenatal or preimplantation testing within 6 hours.

P0426. Strategies And Outcomes Of Over 200 Cycles Of Preimplantation Genetic Diagnosis For Single Gene Disorders

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Couples at high risk of passing on a serious genetic condition to their offspring have the opportunity to use Preimplantation Genetic Diagnosis (PGD) to diagnose a specific genetic disease on embryos obtained through in-vitro fertilisation (IVF) before a clinical pregnancy has been established.

This article reports the experience of our Centre, from 1999 to March 2004, in PGD for single gene disorders (SGD), describing strategies and overall outcome data of 219 PGD cycles performed on fresh cleavage embryos of 156 couples for 22 different genetic conditions. The single gene defect investigated were autosomal dominant (n=13), autosomal recessive (n=127), or X-linked disorders (n=17). Fifty-five

cycles, for 42 couples, were also performed for SGD combined with HLA matching.

A total of 1648 embryos were biopsied, in 1265 (76.8%) of which two blastomeres were removed for analysis. PCR amplification was performed on 2913 blastomeres, obtaining a successful amplification in 2678 (91.9%) cells. Diagnosis was achieved for 1536 (93.2%) embryos, 320 of which were transferred to the patients in 156 of the 219 cycles performed. Forty-seven pregnancies were established (30.0% per transfer). All ongoing pregnancies were confirmed to be unaffected by conventional prenatal diagnosis and went to term without complications, resulting in the birth of 31 healthy babies.

The present results, complementing other similar experiences on PGD, confirm once again the feasibility of the procedure, providing the opportunity for couples who have a known genetically transmittable disease to start a pregnancy with the knowledge that their child will be unaffected with a genetic condition.

P0427. Mosaicism for unbalanced structural autosome rearrangement: better prognosis for male carriers

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Mosaicism for unbalanced chromosome rearrangements is rare and is problematic for genetic counseling when detected prenatally.

Objectives: Analysis of pregnancy outcome in prenatally detected mosaic cases (PDM) according to the type of chromosome abnormality and sex. Comparative analysis of male/female ratio in PDM and postnatally ill-defined mosaic cases (IDM) and in asymptomatic carriers (AM) (transmitting parents, patients with infertility/m miscarriages, fortuitous findings). **Method:** Review of mosaicism for normal line/unbalanced structural rearrangement cases of known sex identified from the literature. **Results:** (1) We identified 99 cases of PDM, from whom pregnancy outcome was reported in 89 cases. Poor outcome was reported in 62% of mosaics for additional material, and in 44% of mosaics for deletion. (2) Males were more frequently reported to be normal (27M/14F), while abnormalities were more common in females (17M/22F). In addition, an excess of females were found to be miscarried, stillborn or dead neonatally (1M/8F). (3) There is an increase in proportion of females in the IDM (57M/100F) compared to the PDM (49M/50F). (4) Seven-fold female preponderance was observed among AM (5M/38F). In contrast, among cases of mosaicism for balanced rearrangements collected during this study, there was no significant female predominance (18M/13F in PDM, 12M/13F in the IDM and 12M/13F in AM). **Conclusions:** The higher incidence of a normal outcome for male fetuses may be the result of sex-specific selection against abnormal cell lines. The strong female prevalence among transmitting parents with gonadal mosaicism can also be explained by such selection rather than by impairment of male gametogenesis.

P0428. The role of genetic counseling on decisions of pregnant women aged 35 years or over regarding amniocentesis in Turkey

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We investigated the effects of genetic counseling given before amniocentesis that is given based on maternal serum screening (using the cut-off value of 1/250) and genetic sonogram results (+/- abnormal ultrasound marker) on pregnant women who are 35 years and older age. Their attitudes towards amniocentesis after genetic counseling were evaluated. Among 340 women, 223 (65.6%) were in the high-risk group and 117 (34.4%) were in the low-risk group according to non-invasive test results. After counseling, 216 pregnant women (167 cases to have high-risk, 49 cases who had low-risk) decided to have amniocentesis while 124 women (56 with high-risk and 68 with low-risk) declined it. Fourteen abnormal karyotypes were detected. All pregnant women who had fetuses with chromosomal aberrations were in high-risk group. Our study shows that screening by non-invasive prenatal diagnostic tool has an effect on families' choice of amniocentesis. The use of these test results during counseling decreased the number of amniocentesis in a ratio of 36.5%.

P0429. Characterization of an unbalanced whole arm translocation leading to monosomy 18p revealed in prenatal diagnosis

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Monosomy 18p is among the most frequently observed autosomal deletions. Terminal deletion of 18p appears as the main reason for occurrence of monosomy 18, while an unbalanced whole arm translocation is reported in 16% of cases. We present a case of monosomy 18p resulting from an unbalanced whole arm translocation revealed in prenatal diagnosis. Amniocentesis was performed on a 41-year old women at 18 weeks of pregnancy, who referred initially for prenatal diagnosis due to advanced maternal age without any USG findings. All metaphases in fetus karyotype analysis revealed an unbalanced whole arm translocation between the long arm of one chromosome 18 and the long arm of one chromosome 22, 45,XX,der(18;22)(q10;q10), which lead to monosomy 18p in the fetus. A de novo origin for der (18;22) was suggested, as the karyotypic analysis of the parents were normal. Chromosome 18p STS markers D18S498, D18S481 and D18S170 were used for genotyping of the fetus and the parents, to determine the origin of der (18;22). Among these, D18S170 marker was informative. Only the paternal allele was detected in the fetus, leaving maternal chromosome 18 to be involved in der (18;22) found.

P0430. Double translocations in prenatal diagnosis

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We report one case of a maternal double reciprocal translocation 46,X,X,t(4;11)(q31;q23),t(5;8)(q35;q22) ascertainment through an offspring with partial trisomy 11q and partial monosomy 4q, karyotype of 46,XX,der(4)t(4;11)(q31;q23),t(5;8)(q35;q22)mat. The second child was healthy 46,XX girl. Cytogenetic analysis of cultured amniotic fluid cells with GTG banding in following pregnancy showed an unbalanced 46,XX,der(11)t(4;11)(q31;q23),t(5;8)(q35;q22)mat karyotype. The parents decided to terminate the pregnancy. The normal karyotype 46,XX was also detected prenatally by amniocentesis in fourth pregnancy.

A second case presents mother with a karyotype 46,XX,t(4;9)(p14;p24), and the father 46,XY,t(1;5)(p11;p11) karyotype. A case of multiple congenital anomalies in a female newborn was found to be associated with partial trisomy 4p and partial monosomy 9q. The karyotype was 46,XX,t(1;5)(p11;p11)pat,der(9)t(4;9)(p14;p24)mat. The unbalanced karyotype was detected prenatally in a fetus by amniocentesis in third pregnancy: 46,XY,der(9)t(4;9)(p14;p24)mat. The pregnancy was terminated. Cytogenetic analysis of cultured amniotic fluid cells in following pregnancy showed double balanced translocation 46,XY,t(1;5)(p11;p11)pat,t(4;9)(p14;p24)mat.

A review of the literature indicates that the risk of having unbalanced offspring is similar in couples in which both parents have a balanced translocation and in couples in which one partner is a translocation carrier. That result from very early spontaneous abortion of mutually unbalanced conceptuses. The fact that the mother with double translocation, and the couple with different balanced reciprocal translocation, has the healthy children indicates the importance of genetic counseling and prenatal diagnosis in cases of double translocations.

P0431. Prenatal diagnosis of autosomal recessive primary microcephaly

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Primary microcephaly (MCPH) is a developmental abnormality resulting in a congenital small-sized but normally formed brain, with mental retardation, and no other neurological deficit. It is usually transmitted as an autosomal recessive trait. Six loci have been reported to date, with ASPM at 1q31 (MCPH5) being most often implicated

in the populations studied. We followed the third pregnancy of a consanguineous couple of healthy, third cousin parents aged 22 and 23 years at birth of their first child, a girl with congenital microcephaly and no other malformation or dysmorphia, with normal skull X-rays and brain MRI except for size. At 2 years her head circumference was 37cm (< 8 SD), height 87.5 cm (p75) and weight 11 kg (p25), and she was diagnosed as MCPH. A second pregnancy was interrupted at the end of the second trimester when microcephaly was detected on ultrasound scanning. DNA analysis in parents, proband, and fetus showed no evidence of autozygosity for known loci except at MCPH5. At 5 years the proband was mentally retarded with no other neurologic deficit, and the couple had a new pregnancy. DNA analysis in amniotic cells showed heterozygosity for linked marker D1S1660 close to ASPM at MCPH5, which we interpreted as an indication that the fetus was probably not affected. Head circumference remained normal at ultrasonography throughout the pregnancy. A normal baby was delivered. We conclude that careful confrontation of linkage and morphometric data may help in prenatal diagnosis of MCPH.

P0432. Prenatal diagnosis of duplication of chromosome 14q resulting from crossover within a paternal pericentric inversion

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We report an extremely rare occurrence of pericentric inversion of chromosome 14 in female fetus with 46,XX,rec14,dup(14q)inv(14)(p11.2q31.2) karyotype. This abnormality was derived from paternal pericentric inversion of chromosome 14. The proposita's partial duplication for the distal segment 14q is apparently the result of crossing-over within the inverted segment during meiosis. All conventional cytogenetic techniques were used to perform chromosome investigation of this structural abnormality. The stillborn, female fetus had various abnormalities. With reference to previous reports, the risk at clinical abnormalities are discussed for both *de novo* and familial pericentric inversions at chromosome 14. To our knowledge, prenatal diagnosis of paternally inherited duplication 14q in a female fetus has not been previously described.

P0433. The single cell as a tool for genetic testing: credibility, precision, implications.

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Tay Sachs disease (TSD) is an autosomal recessive disorder due to impair activity of hexosaminidase A. In its severe form it leads to death during infancy. TSD is frequent among Ashkenazi Jews, with a carrier frequency of 1/29. Two mutations underlie nearly 100% of the infantile form +1278TATC (73% of carriers) and IVS12-1G to C (13%). An adult-onset chronic form, caused by the mutation G805A (4% among carriers) is usually in compound with a common one. Preimplantation genetic diagnosis (PGD) was performed in few cases of couples at risk after terminations of affected pregnancies. Our objective was to measure quantitative aspects of single cell analysis: amplification efficiency and allele-dropout rates in uni, simultaneous (of the two common mutations) and multiplex reactions in: peripheral lymphocytes, fibroblasts (amniotic), mesenchymal (CVS) and blastomeres. A set of nested PCR protocols was designed for the three mutations in different combinations. Each of the protocols can be performed in multiplex with one of two markers flanking the HEXA gene found to be highly polymorphic in the carrier population. Mutations were detected by agarose electrophoresis and two fluorescent computerized genotyping systems. Amplification rate was not significantly different among cell types: 86.3% in lymphocytes (82/95) and 82% in the embryonic cell (64/78). ADO rate was significantly lower (1.3%) in embryonic cells as compared to lymphocytes (12%). Lymphocytes treated with PHA to induce cell-divisions showed a 1.8 fold lower ADO rate than non-dividing lymphocytes. Hypothesis: ADO rate is lower in dividing cells having a less compact nucleus structure.

P0434. Investigation of clastogenic effect of Mitomycin C(MMC) on fibroblast cells(AF, CVS, skin biopsy) in referred patients for prenatal diagnosis of Fanconi Anemia and normal controls

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Fanconi anemia (FA) is a rare autosomal recessive disorder with an extensive genetic and clinical heterogeneity. The affected patients show pancytopenia, many internal and endocrinological problems, as well as high risk of leukemia in particular AML. And the recurrence risk in affected families is 25%. As a result, prenatal diagnosis of FA is an important factor to help such families and the society. Diagnosis of FA on the basis of ultrasound data and biochemical tests is not possible and its molecular diagnosis is difficult and unavailable in most cases. The diagnosis of FA exploits the hypersensitivity of chromosomes of FA lymphocytes and fibroblasts to bifunctional alkylating agents such as mitomycin C (MMC). In this study a cytogenetic test was implicated to access to an accurate diagnosis. During study we have investigated 6 amniotic fluid (AF) referred for FA.. Mitomycin C with the concentration of 2ng/ml have been applied successfully to the fibroblasts of the patients and their normal (negative control) and positive controls .2 fetuses manifested increased chromosome breakage with MMC in their amniotic fluid cells. They were therefore diagnosed as affected Fanconi Anemia.

P0435. Prenatal diagnosis of Osteogenesis imperfecta in Lithuania

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Osteogenesis imperfecta (OI) is a clinically, genetically, biochemically, and radiologically heterogeneous group of inherited connective tissue disorders characterized by bone fragility and other evidence of connective tissue involvement. This definition embraces a very wide range of phenotype, from, at one end, intrauterine or perinatally lethal forms to, at the other end, a barely noticeable increase in fracture tendency.

The form of prenatal diagnostic studies used for a particular pregnancy depends on the nature of diagnostic studies performed earlier and the type of abnormality identified. Four techniques can be used: high resolution ultrasound scan, analysis of the collagen synthesized by cultured chorionic villus fibroblasts, haplotype analysis of fetal DNA, mutational analysis of DNA from CVS or amniotic fluid cells.

Lithuanian OI database comprises 137 case records covering the period of 1980 - 2001. Clinical and genealogical analysis of OI cases/families from Lithuania, available for examination, revealed 17 familial and 23 sporadic cases of OI. As a result of molecular genetic investigation, 13 mutations were identified in the COL1A1 gene. Nine mutations identified in the present study were registered in the Human Type I and Type III Collagen Mutations Database as novel mutations. Prenatal molecular genetic diagnosis is available for 16 families with OI from Lithuania: in three families indirect identification (RFLP-based linkage analysis) of the mutant COL1 locus is possible, while in 13 families COL1A1 gene mutations can be identified directly. Prenatal molecular genetic OI diagnosis enables choosing an optimal strategy for the follow-up of pregnancy and the type of delivery.

P0436. Prenatal diagnosis of limb malformation: towards a better management of patients?

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Limb malformations (LM) are frequent since their prevalence is 1 in 1000 neonates and near 4 in 1000 stillborn. They can be isolated or occur associated with other malformations as part of multiple congenital anomalies syndromes (MCA). Prenatal detection of LM by ultrasound is difficult but helpful to an accurate diagnosis and prognosis. We performed a 4 years prospective study (2001-2004) on

187 limb malformations in the North of France. LM were antenatally diagnosed in 96 cases (51%), straight after birth in 54 cases (29%), and after pathological examination if pregnancy was terminated or in utero death occurred in 37 cases (20%). Results are summarised in the following tables.

96 Prenatal diagnoses				
	Isolated LM: 43		Associated malformations: 53	
Pregnancy	Terminated: 22	Followed: 21	Terminated: 44	Followed: 9
Different LM (after pathological or paediatric examination)	13/22	6/21	26/44	3/9
Associated malformation	6/22	3/21	26/44 (different)	4/9 (different)
Right prenatal diagnosis could change the pregnancy management	2/22	1/21		
LM helpful to accurate diagnosis			32/44	9/9

54 Neonatal diagnoses		
	Isolated: 27	Associated malformations: 27
Associated malformation could have been detected on foetal ultrasound		17/27
Right prenatal diagnosis could change the pregnancy management	1/27	16/17
LM helpful to accurate diagnosis		18/27

37 diagnoses after pregnancy termination or in utero death		
	Isolated: 3	Associated malformations: 34
LM helpful to accurate diagnosis		23/34

CONCLUSION Prenatal detection of limb and associated malformations on ultrasound is very helpful to accurate diagnosis of most MCA syndromes, and should be performed with more fierceness for a better management of patients.

P0437. Twin pregnancy with discordancy for Down syndrome - a case report

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A 29-year-old Caucasian female, pregnant for the second time, underwent ultrasound examination because of her family and obstetric history: first pregnancy ended after 32-week stillborn fetus (normal morphologically appearance but no karyotype was established) and her sister had a baby with trisomy 21. **Objective:** to specify the most appropriate safety method of Down syndrome antenatal screening.

Methods: routine ultrasonography at 7 weeks of pregnancy, selective ultrasonography for the detection of fetal abnormality at 12 weeks, triple test at 15 weeks, amniocentesis - amniotic fluid samples were taken at 16 weeks from each of the two sacs, parental and fetal chromosome analyses. **Results:** ultrasound examinations revealed a twin dizygotic pregnancy with dichorionic placentas; one fetus showed an increased thickness of nuchal fold; biochemical maternal serum investigations revealed low AFP, low uE3 and high HCG levels; lymphocytes from both parents blood were analyzed for chromosomal abnormalities and showed a normal karyotype for the father (46,XY) and a chromosomal translocation for the mother (45,XX, -14,-21,+t(14q21q); the positive results of the tests and maternal chromosomal translocation suggested

us to perform the amniocentesis; fetal chromosomal analysis showed twin 1 with normal karyotype 46,XY and twin 2 with abnormal karyotype 46,XX,+21. After an extensive counseling the parents decided to terminate the pregnancy with Down syndrome. **Conclusions:** first-trimester nuchal translucency scan was a strong and useful screening tool for the prenatal detection of trisomy 21; family history, maternal chromosomal translocation and maternal serum biochemistry were also useful for selective termination of abnormal fetus.

P0438. Multidisciplinary approach to antenatally diagnosed gastroschisis

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Congenital anomalies continue to be the leading cause of infant mortality in the United States. Cause-specific mortality rates have been decreasing, reflecting many factors. But, the rate of serious birth defects continues to be high in economically disadvantaged communities. In New York City, the borough with the highest birth defect rates is the South Bronx, served by Lincoln Medical Center. The obstetric service screens approximately 3,000 pregnant women annually for genetic risk with genetic counseling being provided to about 1,200 of these women.

Between January and July of 2002, there were 1,580 live births and three fetuses were detected with gastroschisis by fetal ultrasound. This translates to a rate of 19/10,000 - more than nine times the expected rate of 2/10,000. Maternal age ranged from 17-20 years. Two of the three mothers had abnormal MS-AFPs, and were Hispanic. All were delivered by cesarean section and no other anomalies were detected. These patients were evaluated by a multi-disciplinary team drawn from Genetics, Neonatology, Obstetrics, and Pediatric Surgery. The etiology and unique issues associated with gastroschisis as well as the special concerns of each family were addressed. This approach, with early detection and thorough patient education, resulted in excellent outcomes for all cases. Aspects of this unusual gastroschisis cluster and optimal psychosocial and clinical management will be described.

P0439. Nucleated Red Blood Cells in maternal blood as a second step in screening for fetal aneuploidies and pregnancy complications

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Nucleated red blood cells (NRBCs) in maternal circulation during pregnancy can facilitate genetic diagnosis either by FISH or PCR analysis, but their rarity and technical difficulties do not as yet allow their use for reliable non invasive prenatal diagnosis. It has been noted however, that an increase of their number could indicate fetal aneuploidies or pregnancy complications.

NRBC number in maternal blood was determined in 173 pregnancies in the 2nd trimester. NRBCs were positively selected by MACS with antiCD71MoAb and identified after anti UcH_y or May Grunwald-Giemsa staining. Among cases with chromosomally normal fetuses (n=119) an average of 8 NRBCs were detected (range 1-12). Among 16 cases with a trisomy 21 fetus the mean number was 71 (22-113) corresponding to a 10-fold increase. 37 NRBCs were isolated from a woman with a trisomy 13 fetus and 52 from a pregnancy with a 45X0 fetus. 64 NRBCs (range 22-158) were identified among 26 women carriers of β-thalassemia trait with normal fetuses. Among 10 women with abnormal Doppler in both uterine arteries 15 NRBCs were isolated (range 2-75). One woman in this group developed PET (6 NRBC) and another delivered an IUGR baby (75 NRBCs).

Determining the number of NRBCs in maternal circulation could represent an additional screening step for fetal aneuploidies as long as the anemic status of the mother is taken into account. More cases with abnormal Doppler must be investigated however before we determine the use of this test in the prediction of pregnancy complications.

P0440. Results of chromosomal study of 2634 amniotic fluids performed in Iran

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Chromosomal study of 2634 amniotic fluid cultures revealed that 2486 (94.4%) had no chromosomal abnormality, 41 (1.6%) had balanced translocation and 107 (4.1%) had chromosomal abnormality. The most common chromosomal abnormalities were Trisomy 21 including free trisomy, Robertsonian translocation and mosaic forms, autosomal derivative chromosomes, Edward syndrome, marker chromosomes, Turner syndrome and sex chromosome mosaic abnormalities, in decreasing order of frequency(1.1%, 0.6%, 0.5%, 0.3%, 0.2% and 0.2% respectively). Sex chromosome abnormalities represented 13 (0.5%) of all chromosomal abnormalities. The reason for referral and their frequencies were high maternal age (1389 cases, 52.7%), history of offspring with chromosomal abnormality (566 cases, 21.5%), fetal loss (530 cases, 20.1%), abnormal alpha-feto-protein in maternal serum (213 cases, 8.1%), parents' reassurance (197 cases, 7.9%), history of offspring with any form of psychomotor retardation (164 cases, 6.2%), maternal chromosomal abnormality (79 cases, 3%), abnormal findings in ultra-sonography (48 cases, 1.8%), abnormal findings in ultra-sonography (48 cases, 1.8%), paternal chromosomal abnormality (42 cases, 1.6%), sex determination (10 cases, 0.4%) and confirmation of the results of chorionic villi sampling (6 cases, 0.2%). 87.4% of the mothers had one indication for chromosomal study 21.1% had two, 1.4% had three and 0.1% had four indications. The rate of chromosomal abnormality for those who had one indication was 3.3%, those with two indications were 5.9%, those with three were 15.8% and those with four were 33.3%. Maternal chromosomal abnormalities, abnormal findings in ultra-sonography and paternal chromosomal abnormalities had the greatest standardized beta coefficients in a multiple regression analysis, respectively.

P0441. The human amniotic fluid proteome

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Proteomic analysis which combines two-dimensional electrophoresis and mass spectrometry has nowadays a wide application in biological and medical sciences, mainly for protein screening in tissues obtained from healthy and diseased conditions, for the detection of drug targets and diagnostic markers. Amniotic fluid samples (AF) are routinely used for prenatal diagnosis of a wide range of fetal abnormalities. In prenatal diagnosis, proteomics have been applied for the analysis of tissues from abnormal fetuses in order to detect differences in the protein profile as compared to the normal one and to determine possible diagnostic tools. A detailed two-dimensional protein database for the normal human AF cells, including 380 different gene products, has already been reported. In the present study we constructed the two-dimensional protein database of the normal human AF supernatant. Ten AF supernatant samples from women carrying normal fetuses were analysed by two dimensional gel electrophoresis. A mean of 412 proteins per gel were analysed and protein identification was carried out by MALDI MS and MS/MS. We constructed a two-dimensional protein map comprising 152 different gene products. The majority of the identified proteins are enzymes, secreted proteins, carriers and immunoglobulins. Twelve hypothetical proteins are also included. The normal AF supernatant proteome map is a valuable tool to study aberrant protein expression and search for new proteins as possible markers for the prediction of abnormal fetuses.

P0442. CVS analysis can be considered the method of choice for rapid cytogenetic prenatal diagnosis. Ten years of experience.

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Chorionic villus sampling keeps its great advantage over amniocentesis due to the early results produced. Recently, quantitative fluorescent polymerase chain reaction (QF-PCR) has entered in the prenatal diagnosis of classical aneuploidies (13, 18, 21, X and Y chromosomes) in amniotic fluid producing extremely rapid results with a detection rate of 98.6%. However, the pathological diagnosis in mid-trimester gestation implying voluntary abortion raises a more difficult situation both surgical and psychological. We present the chorionic villi prenatal diagnoses over last 10 years in our laboratory. We performed 3,868 diagnosis resulting 266 abnormal karyotypes (7%). Among them, 196 were different classical aneuploidies and/or triploidies (73.7%) and 70 (26.3%) presented another chromosomal abnormalities including balanced chromosomal rearrangements. If only QF-PCR had been performed, 26.3% of samples with abnormal karyotype would have been missed and 2% of trisomic cases, which arose from unexpected parental rearrangements would not have benefited from genetic counselling, the unique prevention tool for cytogenetical abnormalities. CVS cytogenetic studies identify a wider range of chromosomal abnormalities than QF-PCR in a few days and the results can be obtained still in the first trimester. Considering the high number of chromosomal abnormalities detected in our series, we think that the CVS analysis is the method of choice for cytogenetic prenatal diagnosis due to its informativity and rapidity.

P0443. First-trimester diagnosis of lethal skeletal dysplasia: transvaginal ultrasound findings

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Over 200 skeletal dysplasias (SD) have been described and around 50% of them are lethal. Current classification of these disorders remains highly complex and molecular basis is yet unknown for many syndromes. Prenatal diagnosis is based on ultrasonography, commonly in the 2nd trimester between 19 and 23 weeks. Ultrasonography is highly specific to predict lethal outcome. However, it is of limited value for identification of the precise bone disorder (41 to 60% identification, according to literature).

This report describes prenatal transvaginal ultrasound findings in 4 cases of SD during first trimester (achondrogenesis 2; thanatophoric dysplasia type 1; short rib-polydactyly syndrome type III Verma-Naumoff; diastrophic dysplasia). One of the 4 cases was a recurrence. All 4 cases were remarkable for enlarged nuchal translucency (3.2 to 6.5 mm) associated with bone abnormalities (femora short or deformed, osteopenia, macrocephaly, extremities or spine malformations). The aim of our study is to emphasize on transvaginal ultrasound performance during first trimester. Overall, sonographers should be aware that enlarged nuchal translucency, when associated with shortness of fetal long bones, may allow detection of lethal SD as soon as 13-14 weeks.

P0444. Prenatal Diagnosis of Exencephaly associated with amniotic band sequence at week 17 by Fetal MRI

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Exencephaly is defined as partial or complete absence of the calvaria (acrania or cranium bifidum) associated with a protrusion of abnormally developed brain tissue and is believed to be an embryological precursor of anencephaly or an encephalocele.

We report a case with exencephaly detected at 17 week gestation on fetal MRI. The mother was a 31-year-old primigravida woman of Asian descent who was referred at 11 weeks of gestation because of an abnormal first trimester ultrasound which suggested an occipital encephalocele. A repeat fetal ultrasound done at 17 week confirmed occipital encephalocele, but could not further elucidate the dimension of the lesion. Prenatal MRI at 17 weeks of gestation showed total absence

of cranial vault with bulging of eyes and the cerebral hemispheres were floating freely in the amniotic fluid. Exencephaly was diagnosed with a small band tethering the hemisphere, suggesting amniotic band. The fetal karyotype was normal (46,XX). Amniotic band sequence is a sporadic condition and can result in a variety of abnormalities including exencephaly. Exencephaly is in most cases a multifactorial condition. Other possible causes include chromosome abnormalities, single gene disorders, maternal diseases and maternal exposures to teratogens. Amniotic band sequence is a rare cause of this abnormality and is difficult to be diagnosed prenatally. To our knowledge there is only one previously reported case of prenatal ultrasound diagnosis of acrania secondary to amniotic band sequence and none documented by fetal MRI. Early fetal brain MRI allows early conclusive prenatal diagnosis and is therefore essential for counselling.

P0445. Chromosomal aberrations as IUGR cause

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IUGR is a pathological condition of pregnancy characterised by birth weight below the 10th centile. A number of fetal, placental and maternal causes can lead to IUGR; although, in most cases no specific causes can be identified. We have analyzed fetal blood karyotype taken by cordocentesis from 168 fetuses with diagnosed IUGR. Chromosomal rearrangements both numerical and structural were detected in 17 cases (11,3%). In three cases inversion 9 (p11; q12) was found; two cases were triploid; trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome) were found in two cases each. There was one case of trisomy 7 and one case of trisomy 16; one translocation (2; 14) (q23; q32) and a deletion 12 (p12) as well as a case of mosaicism 46, XY/46, XX (87,5% / 12,5%). These findings suggest that a consistent number of IUGR cases (about 11%) can be associated with genetic anomalies. Chromosomal aberrations that cause IUGR are heterogeneous, aberration of autosomes, mostly autosomal trisomies, being the most common.

P0446. Distribution of Pvu II(a) alleles at the phenylalanine hydroxylase gene in Republic of Moldova

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Phenylketonuria is an autosomal recessive disease, which results from a severe diminution of the liver enzyme phenylalanine hydroxylase (PAH). More than 10 RFLPs within the PAH locus have been observed in Caucasians population.

On the date of mass neonatal screening we established that incidence of PKU in Moldova is 1:9000 newborns. The RFLP-analysis of Pvu II(a) alleles was performed as described by Dwornicak (1991). The CAG/CTG restriction site of the PAH gene was characterized in 59 families with classical PKU, i.e. 236 parental chromosomes.

Frequencies of normal alleles (see Table) slightly differ from average date in European populations, but not significant ($\chi^2=1,29$; $p>0,5$) and reveals more similarly to the Danish families (0,227/0,773). The level of observed heterozygosity in population of Moldova is 0,37, but the average date in European countries is 0,39. The distribution of mutant PKU alleles in our study not differs significantly from those observed in European ($\chi^2=0,02$; $p>0,08$) and Asian ($\chi^2=0,01$; $p>0,8$) populations. The Pvu II(a) alleles in our populations had a significant difference in the distribution among normal and mutant chromosomes ($\chi^2=7,08$; $p<0,01$). Frequency of informative cases by RFLP-analysis of Pvu II(a) alleles from PKU families is 29% that provide a tool for molecular diagnosis of these disease and carrier status in Republic of Moldova.

Table. Distributions of Pvu II(a) alleles at the PAH gene

Allele	Normal	Mutant	χ^2
E ₁	0,216±0,035	0,093±0,026	7,08; $p<0,01$
E ₂	0,784±0,035	0,907±0,026	

P0447. Prenatal diagnosis of Crouzon Syndrome in the high-risk family

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Crouzon syndrome is an autosomal dominant craniofacial dysostosis. The main features of the syndrome are: craniostenosis, midfacial hypoplasia, proptosis, mandibular prognathism. The syndrome is caused by mutations in the fibroblast growth factor receptor-2 (*FGFR2*) gene. To date, over 30 mutations in *FGFR2* are known. More than a quarter of them are *de novo* mutations.

We present a familial case of Crouzon syndrome diagnosed prenatally by ultrasound examination and confirmed by molecular analysis. Three-dimensional ultrasound examination performed in 27-28 Hbd in the 1st and 2nd pregnancy revealed a deformation of temporal region of the skull and dysmorphic features (ocular proptosis; shallow orbits; wide, flat nasal bridge and prognathism). These signs, together with data from pedigree analysis, have led to suspicion of Crouzon syndrome. The clinical picture assessed after birth varied between the two patients. The analysis of DNA, isolated from amniocytes in the 1st and 2nd pregnancy, revealed the same mutation as found in the father - S267P in 7 exon of the *FGFR2* gene. Presented case is one of only a few prenatally diagnosed cases of Crouzon syndrome and it documents the significance of the modern techniques of prenatal visualization - three-dimensional ultrasonography and magnetic resonance - in the diagnosis of dysmorphic syndromes. Still however, molecular prenatal diagnostics remains the method of choice in high-risk Crouzon syndrome families.

P0448. Screening for large *CFTR* rearrangements should be considered in the diagnostic strategy for CF in fetuses with bowel anomalies

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Recommended investigations when fetal bowel anomalies are detected by ultrasound include fetal karyotyping, screening for infectious disease and screening for frequent cystic fibrosis (CF) mutations. CF is found in France in about 3% of fetuses with bowel anomalies at the 2nd trimester of pregnancy. When a frequent CF mutation is identified in the fetus, the increased residual risk of CF justifies an in-depth study of the second allele by sequential analysis of the 27 *CFTR* exons. Detection of large *CFTR* gene rearrangements using recently developed semi-quantitative fluorescent multiplex PCR assays led to recognize about 20% of previously unidentified CF alleles in various populations of CF patients, which has increased the overall mutation detection rate of diagnostic tools.

We describe a case of fetal hyperechogenic bowel with intestinal loop dilation and absence of gall bladder, a triad highly suggestive of CF. The W1282X mutation was present in the fetus and the father. Sequential analysis of the 27 exons was negative. Screening for large rearrangements led to detect a new deletion removing exons 2-6b in the fetus and the mother and to establish the diagnosis of CF. This case illustrates the need to include the semi-quantitative test in the diagnostic strategy for CF in fetuses with bowel anomalies. Moreover, the method is rapid and sensitive enough to detect micro-deletions/insertions. When applied after testing for frequent mutations, it increases the mutation detection rate of 4.5% in our population, thus being the method of choice for 2nd line screening, especially in emergency cases.

P0449. Six years of preimplantation genetic diagnosis (PGD) for thalassemia, sickle-cell syndromes and cystic fibrosis: a Greek experience

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In Greece β -hemoglobinopathies and cystic fibrosis (CF) have carrier-rates of 10% and 5%. Prevention programmes involving prenatal diagnosis (PND) are well established, but PGD to select unaffected IVF embryos for transfer, is appropriate for some couples. Genotyping of blastomeres from cleavage-stage embryos uses PCR-based methods. However, there are inherent pitfalls, including total PCR failure, allelic drop-out and contamination, which may compromise accuracy of results. Additionally protocols had to be applicable for >25 β -thalassaemia and >80 CF mutations encountered in Greece. Initially we used denaturing gradient gel electrophoresis (DGGE) but now use real-time PCR with multiplexed "mini-fingerprinting" to monitor contamination (Vrettou et al, 2002; Traeger-Synodinos et al, 2003; Vrettou et al, 2004). Over >6 years, 92 couples completed 105 PGD cycles for β -thalassemia and sickle-cell syndromes. Genotypes were achieved in 78% of embryos biopsied, identifying at least one unaffected embryo for transfer in all-but-one cycle. Forty-two pregnancies were initiated, with 14 spontaneously lost. Twenty-one pregnancies produced 29 unaffected babies (8 twins, 13 singletons); PND in 4 on-going pregnancies confirmed 7 unaffected babies; 3 pregnancies await PND. One misdiagnosis (14th PGD performed), was detected by PND and terminated. For CF, 2 couples (of 7 counselled) completed a PGD cycle. Eleven of 14 blastomeres analysed gave genotype result, with 7 unaffected. Transfer of all 7 embryos initiated one singleton and one triplet pregnancy, all confirmed unaffected by PND. Our results demonstrate that PGD for these monogenic diseases is a reliable procedure, well accepted by couples at risk for transmitting β -hemoglobinopathies and CF in Greece.

P0450. Correlation between fetal sex and maternal serum AFP and free beta-hCG during second trimester normal pregnancies

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Objective: To study the effect of fetal sex on maternal serum alpha-fetoprotein (MS-AFP) and free β -human chorionic gonadotropin (free β -hCG) during second trimester of pregnancy.

Methods: The study included 1776 non-smoking women with singleton and uncomplicated pregnancies who were participated in maternal serum screening test for Down's syndrome. The results for both biochemical markers were converted to multiples of median (MoM) for normal pregnancy at the relevant gestational age, and were adjusted for maternal weight. The mean maternal and gestational age were 30.8 ± 5.2 and 16.1 ± 1.4 , respectively. The \log_{10} MoMs of MS-AFP and free β -hCG were compared according to fetal sex.

Results:

Table1. - Comparison of MS-AFP and free β -hCG levels between male and female-bearing pregnancies

	MALES (n=885)	FEMALES (n=891)	P value*
median MS-AFP MoM	0.98 ± 0.34	0.97 ± 0.33	
mean \log_{10} MS-AFP MoM	-0.032	-0.036	0.563
median free β -hCG MoM	1.1 ± 0.66	1.1 ± 0.77	
mean \log_{10} free β -hCG MoM	-0.022	-0.029	0.508

*One-way ANOVA test

Conclusion: No fetal sex differences in the MS-AFP and free β -hCG levels were observed during common term for performing second trimester maternal serum screening test for Down's syndrome.

P0451. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a one-year review prospective experience for the first time in the Czech Republic.

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The increase of maternal age over the last years has intensified the effort to develop early non-invasive methods to screen for trisomy 21

and other chromosomal abnormalities in prenatal diagnosis. In so-called OSCAR clinic (One-Stop Clinic for Assessment of fetal Risk), maternal age, fetal nuchal translucency, as well as maternal levels of free β -human chorionic gonadotropin (β -hCG) and pregnancy-associated plasma protein-A (PAPP-A) are used as screening markers. We report our experience of combining these biochemical and ultrasonographic markers in 686 pregnant women for the first time in the Czech Republic. The specific Down syndrome risk was calculated by using the Fetal Medicine Foundation software. Karyotyping was offered to women with risks ≥ 1 in 250. On the bases of maternal age of the screened population, 1,5 Down syndrome and 1,5 other chromosomal abnormalities were to be detected. 2 out of 2 Down syndrome and 1 out of 1 chromosomal 18 were found resulting in a detection rate of 100 % with a false positive 4,8 % (33/863). The maternal age of the detected cases were 30, 38, 42 years. The population screened showed 18 % aged 35 and more. After the introduction of the first trimester screening to our clinic, the number of invasive genetic testing decreased from 18 % 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.

P0452. Prenatal Diagnostics in Estonia from 1990-2003.

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In Estonia (pop. 1.46 million, birth rate 13082) prenatal diagnosis of genetic disorders was introduced into clinical practice in 1990.

Invasive PND (amniocentesis, CVS, cordocentesis). Altogether 6128 procedures have been done. Abnormalities were detected in 194 (3.2 %) cases. For fetal chromosomal analyses we mostly use amniocentesis (97 %).%)

Altogether 6014 amniocenteses have been done. The main indication (69%) has been maternal age. Chromosomal disorder was diagnosed in 176 (2.9%) cases.

Transabdominal CVS we have used only in high-risk pregnancies in 69 cases. Abnormalities were detected in 14 cases (20%).

Cordocentesis has been done in high-risk pregnancies in 45 cases.

Chromosomal abnormalities were detected in 3 cases (6,6%).

Screening of chromosomal disorders.

Chromosome anomalies have been screened for advanced maternal age since 1995. In 2003, 52 % of women >35 had fetal karyotypes.

Maternal serum screening has been routinely offered since 1998 in Women's Clinic of Tartu, and only in 2002 in whole of Estonia. In 2003, altogether 70% pregnant women under 35 in Estonia were monitored. During the last two years 57% of the trisomy 21(DS) cases were detected prenatally.

Conclusion. Incidence of Down Syndrome(DS) in Estonia after prenatal screening was started has decreased: provisional incidence of DS in Estonia is 1: 660 and after prenatal screening (1995-2003) the incidence of DS is 1: 919 in live birth.

In the future first trimester screening is currently under development in Estonia .

We thank private hospital Ferthal, laboratory HTI and laboratory LTKH for presenting their data.

P0453. Our experience with rapid detection of trisomy 21 using SNP markers.

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Chromosomal abnormalities are the most frequent genetic disorders observed in both live birth and miscarriages. Karyotyping is the most common diagnostic method to detect chromosomal abnormalities. Trisomy 21 is observed in 1 of 800 live births. During the last decade fluorescent-PCR and DNA fragment analysis became also to the tool of rapid detection of gender and most common trisomies. Pont-Kingdon and Lyon (Clin.Chem 49;1087-94,2003) has published first the rapid trisomy 21 detection using SNP allele quantification combined with melting curve analysis. We have established this method in our laboratory using primer-probe system for chromosome 21 SNPs

(WIAF899 and 2643). We have isolated the DNA from 60 trisomy 21 and 49 normal karyotypic samples. PCRs were carried out using Light-Cycler-DNA Master Hybridization Probes (Roche Applied Science) in LightCycler; melting curve analysis was performed following the runs. The whole procedure including DNA isolation and PCR run takes about 2 hours. We found the new trisomy 21 detection system informative with the WIAF 899. There was a significant difference in curve area ratios (0.5234 ± 0.2498 vs. 0.8347 ± 0.099 ; $p=0.001$). We suppose the quantitative PCR with combined with melting curve analysis using selected SNP markers could be an alternative method for detection of trisomy 21.

P0454. Detection of aneuploidies on 5000 prenatal samples by QF-PCR

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QF-PCR analysis of amniotic fluid samples has advantages over cytogenetic analysis. It is fast, detects aneuploidies from minute of samples and does not require viable cells, but it can not detect some chromosome disorders of clinical importance.

Our aims were to test the reliability of QF-PCR for the prenatal diagnosis of the common aneuploidies and to analyze the indications in which amnio-PCR can be applied safely in prenatal diagnosis.

We compared the QF-PCR results with those of the karyotyping of 4985 patients undergoing amniocentesis. We studied the occurrence of chromosome disorders which can not be detected by QF-PCR in cases with different indications of amniocentesis.

98.3% of QF-PCR results were informative without false-negative and false-positive results. 126 chromosomal abnormalities of 152 were detectable by QF-PCR. QF-PCR detected all aneuploidies in case of advanced maternal age. In case of structural abnormalities detected by ultrasound, the chromosome disorder was not detectable in 23 cases. We applied a reliable, simple and cost-effective QF-PCR protocol using 7 STRs. It is a reliable alternative to karyotyping in case of advanced maternal age, but less reliable in case of first and second trimester ultrasound markers. Prenatal multiplex QF-PCR diagnosis of trisomies 21, 18, 13 and sex chromosome anomalies is reliable, but the indication of the prenatal diagnosis should be considered prior its application.

P0455. Hartsfield holoprosencephaly-ectrodactyly-clefting syndrome: further delineation and review

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The association of ectrodactyly, holoprosencephaly and clefting was first described in 1984 by Hartsfield et al. In 20 years, only 7 patients have been reported. We present a male fetus, third child of an healthy unrelated couple. Ultrasonography at 24 weeks of gestation revealed ectrodactyly and cleft lip, suggesting an EEC syndrome. Brain MRI performed at 32 weeks of gestation showed vermis hypoplasia. After termination of pregnancy, examination of the fetus showed bilaterally hypoplastic third fingers with absent nails and cleft feet with only 3 toes, left labial and gingival cleft without cleft palate, and mild hypertelorism. Neuropathologic examination disclosed short vermis and arhinencephaly.

The present case appears to belong to Hartsfield syndrome (HS) spectrum. Its hallmarks are ectrodactyly and holoprosencephaly, unusual hypertelorism, cleft lip and palate, abnormal, low set ears, and in some cases, hypernatremia and gonadotropin deficiency. Surviving children have severe mental retardation. HS has only been reported in single male fetuses. Arhinencephaly which is part of holoprosencephaly spectrum was present in 2 of these cases, but vermic hypoplasia was not reported before. Genetic basis of HS are unknown. XLR inheritance is a possibility. Mutation screening of p63 and SHH are pending.

This rare observation stress the usefulness of fetal brain imaging in differential diagnosis of syndromal clefting diagnosed in utero, particularly when EEC is suspected.

P0456. Study of maternal and paternal age in 2634 chromosomal studies of amniotic fluid

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We studied the age of the mother and father in 2634 amniotic fluid chromosomal studies. We determined the distribution of chromosomal abnormality in the fetus according to the maternal and paternal age. Age groups with a ten year interval were determined and the frequency of each group determined for both parents. The frequency of chromosomal abnormality of the fetus was determined for each age group. The age group with the highest rate of chromosomal abnormality in the fetus was 35-44 years for the mothers and 30-39 years for the father. The mean (\pm SD) and median of mothers' age were 33.8 ± 6.2 and 35 years (ranged from 15 to 48 years) and for fathers were 38.0 ± 7.0 and 38 years (ranged from 20 to 76 years), respectively. The mean maternal and paternal age (\pm SD) of parents of fetuses' with chromosomal abnormality were 32.8 ± 6.2 and 36.8 ± 7.2 . When we excluded the parents with chromosomal abnormality the maternal and paternal age increased to 35 and 39. The mean age of the mother with a fetus with Down syndrome was (36.5), Edward syndrome(36.5) and Klinefelter syndrome(39.5) which were higher than the mean age of mothers with normal fetus. However the mean maternal age was significantly lower in Turner syndrome (29). The mean age of mother's with a fetus with an extra marker chromosome was similar to the normal group. The mean paternal age was higher than the normal group in Down syndrome(39.3) and Edward syndrome(43.5), but lower in Turner(31.4), Klinefelter(35.5) and fetuses with a marker chromosome(33.4).

P0457. Study of consanguinity in parents of 2600 amniotic fluid samples

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Consanguineous marriages are common in our country, ranging from 15-25%. In spite of the prevailing policy to educate couples to avoid consanguineous marriages, many couples believe that performing an amniocentesis and chromosomal study, it can prevent the birth of a congenitally abnormal offspring. In our study we confirmed that there was no increased risk of chromosomal abnormality in related couples. Consanguinity was studied in parents of 2600 parents of amniotic fluid samples. 79% of the parents were not related and 21% were related. The inbreeding coefficient was greater or equal to 1/16 in 429 (16.5%) and less in 128 (4.9%). Reasons for referral for amniotic fluid chromosomal study were as follows: high maternal age, history of offspring with chromosomal abnormality, abnormal alpha-feto-protein in maternal serum, abnormal findings in ultra-sonography, fetal loss, history of offspring with psychomotor retardation, sex determination, chromosomal abnormality in parents and reassurance. Cases with high maternal age had the highest frequency of related parents (29.6%) and those with fetal loss had the least (17.8%). The frequencies of related parents in the other cases were similar and ranged from 19.5-22.1%. The frequencies of chromosomal abnormalities in related and unrelated parents showed no statistically significant difference (3.6% vs. 4.2%, $p=0.513$). The frequency of Down syndrome was greater in unrelated parents 1.4% vs. 0.2%, $p=0.018$). The frequency of Edward syndrome and Turner syndrome were 0.5% and 0.2% in the unrelated parents, respectively, and 0.2% and 0.2% in the related group ($p=0.268$ for Edward, $p=0.477$ for Turner syndrome).

P0458. The importance of cooperation between geneticist and gynaecologist; results of prenatal genetic diagnostics in the year 2004; interesting patients.

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Our hospital was the first place where the prenatal genetic diagnostics (PGD) start in Slovak republic in 80 - years. We point to close cooperation of geneticist and gynaecologist in our work. In 2004 we indicate 490 patients to PGD at our department. We cytogenetically analyzed 487 samples of amniotic fluid. Successful rate of cultivation was 98,1%. Gynaecologic clinics that cooperates with us performs consiliar ultrasound (US) examinations so that is the reason, why the ultrasound pathology is so frequent in our set.

Indication groups	Number (n)	Karyotype normal/patho-logic	Termination of pregnancy
Age over 35 y.	270	256/2	2x
Bioch.screening - chrom. aberrations	97	91/5	5x
Bioch.screening - NTD	58	50/0	
Inborn develop. defect in anamnesis	6	6)0	
Pathologic US result	34	34/6	16x 1xSAb
Chr.aberration in anamn.	25	25/0	
Reprod.failures in anamn.	5	5)0	
DNA dg	10	7)0	
Others	11	11)0	
Σ	516#	487/13	23x 1xSAb

the total amount of diagnoses is bigger then number of indicated patients (combined indications to PGD)

According to the examinations the special obstetrician management is indicated, that includes delivery in the hospital, where the specialised neonatologic intervention is possible. In cases when the termination of pregnancy is realized, geneticist makes observation of the fetus, verification of prenatally postulated karyotype is done and pathological examination is indicated. This management is necessary for determination of exact etiology and prognosis. At the end of our presentation we demonstrate two interesting patients.

P0459. Contingent strategies for Down's syndrome screening

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Screening sequentially across both the first and second trimester can achieve a higher Down's syndrome detection rate than within-trimester protocols. Among sequential strategies, by far the most efficient is contingent screening. This involves restricting second trimester tests to those with borderline Down's syndrome risks based on the maternal age and first trimester marker profile. Those with very high risks are offered immediate invasive prenatal diagnosis, those with very low risks are reassured and the remainder are classified as screen-positive or screen-negative from their final risk based on all first and second trimester markers combined. Statistical modelling can be used to predict the detection and false-positive rates of the different strategies. For example, a contingent protocol of first trimester maternal serum pregnancy-associated plasma protein-A and free beta-human chorionic gonadotropin (hCG) together with ultrasound nuchal translucency (NT) followed by second trimester serum alpha-fetoprotein, free beta-hCG, unconjugated estriol and inhibin-A could yield a 94% detection rate for a 5% false-positive rate. This can be achieved with 70% of Down's syndrome pregnancies detected in the first trimester and selecting only 15% of women for second trimester tests. Contingent screening might also be considered within the first trimester for localities with limited ultrasound facilities: NT would be restricted to 20-30% of women selected by risk based on age and serum markers. This approach could be extended with a further contingent step to select for even more specialist scans for nasal bone hypoplasia and ductus venosus. Moreover, the within-trimester and sequential contingent approaches can be combined.

P0460. The incidence of cystic fibrosis in second-trimester fetuses with hyperechoic bowel

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Objective. To determine the significance of fetal hyperechogenic bowel as a specific ultrasonographic marker for the cystic fibrosis in the low-risk pregnant women population.

Methods. Sixteen fetuses with the echogenicity of bowel of surrounding bone were included in our study. Genomic DNA was obtained from amniocytes and fetal blood. LightCycler-based fluorescent-labeled oligonucleotide melting assay use the fluorimeter to screen DNA samples for the cystic fibrosis mutations. The family history was negative in all cases regarding to cystic fibrosis and bowel disease.

Results. The mean gestational age was 19.2 weeks ranging from 17 to 26 weeks of gestation. The screening test for cystic fibrosis transmembrane conductance regulator mutations was performed in all included cases. One fetus was found to be a heterozygote carrier for ΔF508 mutation. The presence of echogenic dilated bowel loops was noted in one fetus homozygous for ΔF508 mutation. The incidence of cystic fibrosis was 6% in our study group. The termination of pregnancy was performed based on the abnormal result of cystic fibrosis mutational analysis.

Conclusions. The appearance of isolated hyperechoic bowel in the second trimester fetuses was found to be associated with a significantly higher risk for cystic fibrosis. The finding of specific ultrasonographic marker indicates the necessity for further prenatal molecular testing, which is of critical importance in low-risk population.

P0461. Carrier detection and prenatal molecular diagnosis in Duchenne muscular dystrophy in Iranian families

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Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive disorder affecting 1 in 3500 male births. It is caused by mutations in the DMD gene, located on Xp21.2. Two-thirds of the patients have large intragenic deletions or duplications and the remaining one-third have point mutations, small deletions or insertions. Mutations in the DMD gene result in a progressive muscle degeneration and early death. The direct mutation identification in female relatives of DMD patients was done using multiplex exon amplification by PCR. Also, linkage analysis using three intragenic RFLPs and two main CA repeats was performed. During the last five years, carrier detection was performed for 118 families. Our data indicates that deletions were found in 53.1% of the patients, with 77.6% of the deletions confined to the distal hot spot and 22.4% of the deletions related to the proximal hot spot. When female carriers were detected, prenatal molecular diagnoses were performed in DMD families. Prenatal diagnosis in 34 families, including 21 male and 13 female fetuses, revealed that 5 of the males were affected and 16 were normal. Four of the female fetuses were carrier and the remaining cases were normal. We also performed three intragenic RFLPs for 81 families. In 51 families, one or more of RFLPs were informative (56.2%). In the remaining families, RFLPs were not informative. The most informative RFLP was BamHI and the least was TaqI. Microsatellite analysis on 38 families was informative in 27 families. The most informative STR was STR49 and the least was STR45.

P0462. Early prenatal diagnosis of triploidy and nuchal translucency measurement in the first trimester of pregnancy

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Triploidy - one of frequent chromosome anomalies at early periods of pregnancy, revealing significant deflections in development of embryo and chorion. In ditto time, there was described cases of births alive children with this severe pathology. Well-timed discovery

and interruption of such pregnancy is important medical and social problem.

Objectives: Study the ultrasonic markers 1 trimester, especially measurement nuchal translucency, in triploid embryos.

Study design: We have analysed the cases triploidy, revealed in the Centre geneticists of Republic Tartarstan during 2001-2003 years.

Results: There was examined on the same ultrasound technic strategy 4980 women from 11 to 14 weeks of pregnancy, amongst which there was revealed 6 embryos with triploidy, confirmed following invasive procedure (the chorion villies samples or cordocentesis). All confirmed pregnancy was interrupted.

Conclusions: There is strong effectiveness of nuchal translucency measurement as a screening tool for the early prenatal detection of triploidy.

P0463. Evidence of blood chimerism in a spontaneous dizygotic twin pregnancy discordant for trisomy 21

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A monochorionic-diamniotic placenta (MCDAP) is rare in dizygotic twinning. All reported cases have been documented in IVF pregnancies. Consequently, this phenomenon was thought to be inherent to IVF. We report a spontaneous pregnancy in a 39-year-old patient with evidence of MCDAP in dizygotic twins, discordant for trisomy 21. First and second trimester sonographic scans indicated male twins with MCDAP. Amniocentesis, performed due to advanced maternal age, revealed a normal karyotype in one fetus, and trisomy 21 in the other. Molecular studies, performed in order to confirm the zygosity and chorionicity, demonstrated that the fetuses were dizygotic. In order to identify the affected twin, a detailed sonographic examination was repeated, but no abnormal findings associated with Down syndrome were demonstrated in any of the fetuses. Therefore, umbilical cord blood samples were obtained from both fetuses. Chromosomal analysis revealed in both fetuses two cell-lines: a normal cell-line of 46,XY and a 47 XY +21 cell-line, in 65% and 80% of the cells, respectively. This result was independently confirmed by both FISH and G-banding. DNA extracted from both cord blood samples, demonstrated an admixture of 2 distinct genotypes in each sample. We propose that this case represents a monochorionic-dizygotic twin pregnancy with blood chimerism. The most plausible mechanism underlying this phenomenon is that of placental fusion in early pregnancy, resulting in an architecturally single placenta originating from two distinct zygotes. The newly formed blood vessels created anastomoses between the dizygotic twins and allowed reciprocal blood chimerism between the normal and the trisomic twins.

P0464. Volume of sampled amniotic fluid and prenatal cytogenetic diagnosis: results of retrospective study

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OBJECTIVE: To assess the relationship between amniotic fluid sample volume, culture time and culture failure in prenatal diagnosis.

MATERIALS and METHODS: We retrospectively evaluated the results of 225 cases of genetic amniocentesis performed between 16 and 20 weeks of gestation. Cases were separated into four groups according to the amount of amniotic fluid obtained: group 1, 1 to 5 mL (n=12); group 2, 6 to 10 mL (n=23); group 3, 11 to 15 mL (n=10); and group 4, 16-20 mL (n=180).

RESULTS: Culture time was significantly longer in group 1 than in the

other groups. (median 19 days vs. 12.5, 10, 11 days). Even if differences in culture time between groups 2 to 4 were not significant, there was a trend towards an increase in culture time with decreasing amniotic fluid volumes. Moreover, risk of culture failure was also increased with smaller volumes. There were no culture failures in groups 2, 3 and 4, but two culture failures in group 1.

CONCLUSION: When necessary, small amniotic fluid volumes may be used for fetal karyotyping after information of the family. Small volumes of amniotic fluid increase culture time, rate of culture failure and cost of culture.

P0465. 2nd trimester ultrasound diagnosis of hypophosphatasia in a low risk pregnancy due to homozygous mutation 1130C>T in the tissue nonspecific alkaline phosphatase gene

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Hypophosphatasia is an inherited bone demineralizing disorder caused by missense mutations in tissue nonspecific alkaline phosphatase (TNSALP) gene. The clinical appearance varies widely from a lethal form (perinatal) to a milder adult-onset form (odontohypophosphatasia). So far 127 TNSALP gene mutations have been associated with hypophosphatasia. A few prenatal diagnoses of hypophosphatasia have been made, all in high risk pregnancies. We believe this is the first diagnosis of hypophosphatasia in a low risk pregnancy made, on the basis of the US scan, in a G₃ P₂ 28-year-old caucasian woman earlier than the 28th gestational week. The patient was referred to our tertiary centre because of a diagnosis of skeletal dysplasia. The 2D scan showed a single living fetus with generalized demineralization of all bones. Ribs were shortened and fractured, mesomeric segments of the four arms appeared shorter and curved with evidence of epiphyseal osteolysis, rizhomeric segments appeared markedly hypoplastic. The fetus appeared hydropic. Hydramnios was present. The presumptive diagnosis was made on the detection of completely demineralised calvarium which seems to be a selective US marker for hypophosphatasia. The fetus, delivered at 35 weeks by a caesarean section, died after a few hours owing to respiratory failure. Dysmorphologic, X-Ray and necropsy examination reinforced the suspicion of hypophosphatasia, which was confirmed by sequencing the TNSALP gene, showing a 1130C>T homozygote mutation. The parents were shown by sequencing to be carriers. Accurate US scan is a useful tool for prenatal diagnosis of hypophosphatasia.

P0466. Prenatal diagnostics of congenital malformations and hereditary diseases in Bashkortostan Republic of Russia

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Prenatal diagnostics of congenital malformations and chromosomal aberrations in Bashkortostan Republic of Russia is carried out using alpha-fetoprotein screening, ultrasonographic diagnostics of pregnant women. Medical Genetic Center provides the second phase of prenatal screening and implements invasive methods including chorion biopsy, placentocentesis, amniocentesis, and cordocentesis. The frequency of congenital malformations in Bashkortostan Republic is 8.1 per 1000 newborn children. The most frequent malformations are multiple congenital malformations, Down's syndrome, cleft palate. Ultrasonic diagnostics reveals 22-34% of all congenital malformations. Karyotype anomalies have been found in 871 cases from 2064 children with congenital malformations. In 20 cases prenatal diagnostics has been carried out in patients at risk with phenylketonuria, Huntington's disease, Duschenne-Bekker myodystrophy, cystic fibrosis, hemophilia, adrenogenital syndrome. Genetic registers for the seven most spread monogenic diseases have been created and are used in medical genetic consultation.

P0467. Fetal RHD genotyping by maternal serum analysis: a two-year experience

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Fetal RHD genotype determination is useful in the management of RhD-negative women, avoiding particularly the unnecessary administration of anti-D immunoglobulin (a blood derivative) in adapt prophylactic anti-D immunoglobulin infusion in case of a RhD-negative fetus. The discovery of fetal DNA circulating in the serum of pregnant women offers new opportunities and non-invasive determination of fetal RHD genotype has become reality.

We report a two-year experience with fetal RHD genotyping by maternal serum routinely proposed in a prenatal diagnosis center. After informed consent, 285 women elected to participate accepted blood sampling; all were Caucasian but none were allo-immunized. The mean gestational age was 15.2 weeks (range: 8-35).

In two cases the status of the fetus could not be determined (RhD-negative phenotype of the mother not in relation with a complete RHD gene deletion). Fetal RHD genotype could be determined from all other 283 maternal sera; in 179 cases the fetuses were considered as being RhD-positive while the others (n=104) were RhD-negative.

These results could be controlled for 272 patients either by analysis of amniotic fluids and/or by serological study of the newborn (11 lost to follow-up) and were in complete concordance. All sera from women carrying a RhD-positive fetus (n=170) gave positive results for RHD gene detection while all sera from women carrying a RhD-negative fetus (n=102) gave negative results.

Fetal RHD genotype can be determined with a high level of accuracy by analysis of fetal DNA circulating in maternal serum and could be included in prenatal care of RhD-negative women.

P0468. Identification of a novel de novo PAX2 mutation in a fetus presenting asymmetric renal involvement and optic nerve coloboma

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The renal-coloboma syndrome (MIM 120330) is an autosomal dominant condition including clinically optic nerve coloboma and interstitial renal fibrosis, nephritis or renal failure. Eccles reported that some cases have vesicoureteral reflux, high-frequency hearing loss, and/or genital anomalies, while central nervous system anomalies have been occasionally reported. These clinical signs are consistent with the expression of PAX2 in these tissues during development.

Termination of pregnancy was elected at 24 weeks for anhydramnios and suspicion of renal agenesis in the healthy couple with unremarkable previous clinical history. At autopsy fetus presented sequence of external features related to oligo-anhydramnios including Potter gestalt, and multiple arthrogryposis. Internal examination revealed bilateral kidney anomalies including small multicystic left kidney (1.8g), and extremely hypoplastic right kidney (0, 34g). Histology showed highly dysplastic lesions in the left kidney, contrasting the normal organisation in however hypoplastic right kidney. Ocular examination disclosed optic nerve coloboma. The association of those anomalies highly suggestive of renal-coloboma syndrome led us to perform molecular study of PAX2 gene.

Direct sequencing of the PAX2 coding sequence identified a single G deletion of nucleotide 935 in exon 3 of the PAX2 resulting in a frameshift mutation (131fsX158).

The parents underwent ultrasound kidney examination which was normal in both.

This is the first diagnosis of Renal-Coloboma Syndrome in a fetal case based on the kidney and ocular histology, and another example of impact of oriented fetopathological examination in genetic counselling of the parents.

P0469. Evaluation of relationship between ductus venosus and umbilical artery blood flow, nuchal translucency, maternal serum PAPP-A and free beta hCG in the first trimester.

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Evaluation of fetal ductus venosus waveform is important in the assessment of cardiovascular system. The objective of the present study was to investigate the ductus venosus and umbilical artery waveforms in first trimester fetuses and to correlate them with nuchal translucency thickness and maternal serum PAPP-A and free beta hCG subunit concentrations. METHODS: Fetuses between 10 and 13-14 weeks of gestation were consecutively enrolled into the study. The analysis comprised the following parameters: ductus venosus pulsatility index for veins, presence of A wave, umbilical artery resistance indices and maternal serum parameters. The Doppler and biochemical values obtained were plotted on reference ranges. RESULTS: 194 fetuses were included in the study. We observed a significant inverse correlation between PAPP-A and umbilical artery resistance and a borderline negative relationship between the pulsatility index for veins in the ductus venosus and PAPP-A concentration. CONCLUSIONS: The umbilical artery resistance is negatively correlated with maternal serum PAPP-A levels.

P0470. Congenital perisylvian polymicrogyria in four akinetic fetuses

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Congenital Perisylvian Polymicrogyria (CPP) is reported in four male fetuses, medically aborted for prenatal ultrasonographic diagnosis of akinesia.

Methods. Complete post mortem studies were performed including skeletal X-rays, brain ultrasonography and extensive fetopathological examination.

Results. All fetuses shared external features of the dyskinesia deformation sequence. Karyotypes on amniotic fluids were normal. Microscopic examination of the brain revealed a bilateral unlayered polymicrogyria, surrounding the sylvian fissures. At the spinal cord level, the motor neuronal population was reduced or absent, replaced by gliotic tissue. Extraneurological lesions, matching with prenatal ultrasound findings, were consisted of scattered calcifications within the liver in two cases and within the myocardium in one case, with a membranous interventricular septum defect. Excess of basal fibrin deposits was present in the placenta in one case.

Conclusion. CPP is a rare malformation of the cortical plate, characterised by a bilateral abnormal gyral pattern in the sylvian regions that may be responsible for developmental and neurological problems. An X-linked inheritance, suggested by the recurrence pattern in some families, is supported by the discover of a locus at Xq28. A spinal cord involvement in CPP has been evocated in children with a club feet deformity but a few cases are documented in association to fetal arthrogryposis. Concerning the pathogenesis, CPP has been attributed to hypoxic-ischemic insult in the middle cerebral artery territory. Similar transient vascular injury may also have occurred in the spinal cord and in viscera during the gestation.

P0471. Prenatal diagnosis of trisomy 21 by de novo robertsonian translocation t (21;21).

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Case report: We report a fetus who had robertsonian translocation with 47,XX+21,t(21;21) karyotype. A 36 year old gravida 1, para 0 woman was referred to our Genetic Department for genetic counselling at 16 weeks gestation due to advanced maternal age. Ultrasound scan demonstrated increased nuchal thickening (0,65 cm cervical-occipital fold). Genetic amniocentesis performed upon those findings revealed a 47,XX+21,t(21;21). 45/45 amniotic fluid cells were trisomic and detected in 3 independent cultures. After detailed genetic counselling parents decided to have a therapeutic abortion, which was performed at 20+2 weeks of gestation. The post abortion examination revealed

a dysmorphic phenotype and the cord blood culture confirmed the prenatal diagnosis. Both parents had normal blood karyotype. Causes and mechanisms of development of de novo robertsonian translocation in our case are discussed.

Conclusions: Pathological ultrasonographic results were highly correlated with chromosomally abnormal fetus. In our opinion in all cases with abnormal ultrasonographic findings amniocentesis must be performed. Fetal karyotyping is important in counselling and in pregnancy management implications.

P0472. Retrospective analysis of a series of fetuses referred for FGFR3-related skeletal dysplasia in Greece: report of two hypochondroplasia cases

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Molecular genetic screening for detection of mutations in the FGFR3 gene related to achondroplasia, hypochondroplasia and thanatophoric dysplasia type I and II, was performed in a cohort of 65 fetuses of Greek origin with ultrasound findings consistent with skeletal dysplasia. Preliminary results include the detection of the most common achondroplasia mutation in one case and hypochondroplasia mutation in two cases. Reports of prenatal diagnosis of hypochondroplasia are very rare and the phenotype/genotype correlation in these cases is poor. Hypochondroplasia is an autosomal dominant skeletal dysplasia characterized by micromelia, short stature and lumbar lordosis, exhibiting a phenotype similar but milder compared to achondroplasia. Molecular genetic analysis revealed that both fetuses were heterozygous for the C1620A mutation resulting in N540K substitution in FGFR3 gene. We conclude that the combination of ultrasound and molecular genetic approach is crucial for establishing an accurate diagnosis of hypochondroplasia *in utero* and subsequently for appropriate genetic counselling and perinatal management. To our knowledge this is the first study in Greece in which the assessment of genetic and prenatal ultrasound data led to the differential diagnosis of hypochondroplasia.

P0473. Evaluation of rapid prenatal diagnosis by QF-PCR in 28.000 consecutive prenatal samples

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The Quantitative Fluorescent PCR (QF-PCR) assay, introduced during the last few years, allows performing prenatal diagnoses of common chromosome aneuploidies in a few hours after sampling.

In this study we report the evaluation of QF-PCR tests performed on a large cohort of 28.000 consecutive clinical specimens analyzed in two different Centers. Results were compared with conventional cytogenetic analysis. QF-PCR detected 26.768 normal fetuses. No false positives were observed. A total of 1123 abnormal karyotypes were detected by cytogenetic analysis, 1036 cases were correctly diagnosed by QF-PCR as due to trisomy 21, 18, 13, triploidies, double trisomies and aneuploidies of the XY chromosomes. Four partial trisomies were also identified together with 27 cases of chromosome mosaicism; aneuploidy detection was only hampered by maternal contamination or low level mosaicism. In 22 cases of culture failures, QF-PCR was the only evidence of fetal X, Y, 21, 18 and 13 chromosome complement. QF-PCR proved efficient and reliable demonstrating 93% overall sensitivity; the assay detected 95% of clinically significant chromosome abnormalities with 100% specificity, PPV of 100% and NPV of 99.8%. The assay reaches the purposes of relieving anxiety of most parents within 24 hours from sampling or to accelerate therapeutic interventions in case of abnormal result. Main advantages of QF-PCR are its very low cost, speed and automation, enabling to analyze more than 100 samples per day. In countries where cytogenetic analysis is hampered by its high cost and lack of technical expertise QF-PCR may be used as the only prenatal diagnostic test

P0474. The analysis of first year activity in chromosomal prenatal diagnosis laboratory in Iasi, Romania

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We analyzed the first year of activity of cytogenetic prenatal diagnosis laboratory from "Cuza-Voda" Hospital Iasi, Romania. Our laboratory make the analysis by FISH method after amniocentesis. The reasons for amniocentesis were: maternal age - more 35 years (7 cases) sonographic abnormalities (21 cases) a child with Down syndrome in pedigree (4 cases) an abnormal triple test (1 case) and stopped pregnancy (4 cases). We discovered 9 pathological cases (24.32%): 18 trisomy mosaicism (5 cases), 18 trisomy homogenous (1 case) 21 trisomy (2 cases) and a 46,XX/46,XY formula (1 case). The correlation between reason for amniocentesis and chromosomal result was: maternal advanced age - 2 cases (18 trisomy); sonographic abnormalities - 5 cases (18 trisomy- 3 cases; 21 trisomy - 2 cases); stopped pregnancy - 1 case (18 trisomy homogenous); and a child with Down syndrome in pedigree - 1 case (46,XX/46,XY formula). The results was confirmed by pathological examination (5 cases ended by abortion) or by clinical examination and postnatal karyotyping (2 cases). In 2 cases (one is 46,XX/46,XY) the pregnancies are not finished, and we will wait the birth for supplementary examinations. The presence of 6 cases with 18 trisomy against 2 cases with 21 trisomy, can be explained by presence of more severe malformations in 18 trisomy. In conclusion our study reveals the importance of chromosomal prenatal diagnosis and the necessity of clinical and paraclinical trial at women that make amniocentesis.

P0475. Prenatal karyotyping of fetuses conceived by intracytoplasmatic sperm injection (ICSI)

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The objective of our study was to determine the incidence of chromosomal anomalies in a cohort of ICSI pregnancies. We examined 146 singular, 22 twin and 1 triplet pregnancies conceived by ICSI. The patients underwent genetic amniocentesis or CVS and cytogenetic analysis. We analysed the frequency of chromosome abnormalities and their relationship to parental age. The average maternal age was 34.6 ± 3.7 years. Amniocentesis/ CVS and karyotyping was performed to evaluate the 193 fetuses from 169 ICSI pregnancies. Intrauterine karyotyping showed 5 cases of chromosome disorders out of the 146 singular pregnancies (3.4%). Three cases of trisomy 21, one case of trisomy 18 and one case of X monosomy were found. Out of the 22 twin pregnancies we found one case of trisomy 21 (4.45%), while in the triplet pregnancy all three fetuses had normal karyotypes. In case of maternal age over 35 years, we found 3 aneuploid fetuses of 80 patients (3.7%). In case of mothers younger than 35, we found 3 aneuploid fetuses of 89 patients (3.4%). We did not detect abnormal karyotype from the parents of the affected fetuses. The ratio of chromosomal abnormalities seems to be slightly increased in ICSI pregnancies. We found no fetal aneuploidy in case of paternal age over 45. The incidence of aneuploid fetuses was not significantly increased in cases of mothers aged over 35. Our observation supports the need for fetal chromosome analysis of fetuses conceived by ICSI.

P0476. Prenatal Diagnosis of Spinal Muscular Atrophy (SMA) in Iranian families

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Spinal muscular atrophy (SMA) is a common neuromuscular disorder with progressive paralysis caused by the loss of α - motor neuron in the spinal cord. The SMA candidate interval genes are located on the long arm of chromosome 5 (5q13) on two highly homologous copies (telomeric and centeromeric) within the SMA region. Homozygous disruption of SMN1 (survival motor neuron) is the major cause of SMA, while SMA severity is mainly determined by another genes including; NAIP (neuronal apoptosis inhibitory protein), P44 and copy number of SMN2. SMN1 is the SMA determining gene disrupted in more than 90% of SMA cases. In this study, SMN1gene was analyzed in

amniotic fluid or chronic villous sampling (CVS) samples of 46 Iranian SMA Families, in which at least one affected child was observed. We found 19% (9/46) SMN1gene homozygous deletion in fetus samples and 56% (26/46) of them was carriers for SMN1 gene.

P0477. Assessment of the effectiveness of combined ultrasound and biochemical screening of fetal chromosomal diseases in the first trimester of pregnancy.

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Assessment of risk of fetal chromosomal diseases proceeds due to maternal age, concentration of PAPP-A, free β -hCG, value of nuchal translucency (NT) and presence/absence of nasal bone. It is very important to make such analysis due to lack of such investigations in Russian literature. We investigate 1025 women at 10-13 weeks of pregnancy, mean age 32 (16-47). Among all women 22% was over 35 years old. We use ultrasound scanner Aloka SSD-2000 and system of prenatal screening «Life Cycle» (Pribori Oy, Moscow). In 40 of 1025 fetuses there was NT 2,5 mm or more and 17 of them have chromosomal diseases. In 4 of 1025 fetuses there was absence of fetal nasal bone, 3 of them have chromosomal diseases and one has severe malformations. In 109 of 1025 patients there was increased risk of fetal chromosomal pathology due to results of biochemical screening, 13 of them have chromosomal pathology. Totally we diagnosed 21 out of 22 fetuses with chromosomal diseases (8 fetuses with trisomy 21, 3 fetuses with trisomy 18, 6 fetuses with Turner syndrome, one of each with trisomy 13, 47,XXY, 47,XXX and triploidy). Detection rate of combined screening in the first trimester of pregnancy was 95,4%, sensitivity and specificity was 95,4% and 88,7%, false positive rate - 11%. High false positive rate in our results can be explained due to high percent of women over 35 years in our study.

P0478. Use of plasmid calibrators to quantify fetal and total DNA in maternal blood : application to Trisomy 21 affected pregnancy.

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Background: Quantification of free DNA in maternal blood is a potential marker of genetic disorders and complications of pregnancy. Each laboratory generate data under specific conditions highlighting the need of procedure standardisation, first step to an essential multicentric study. We propose tools and a methodology for standardization of the quantification of free DNA in maternal blood and its application in Down syndrome pregnancies.

Methods: We have based on an original approach of calibration which consists in a standardized procedure and manufacturing plasmid DNA calibrators containing the target gene sequence: β -globin for total DNA and SRY for fetal DNA in order to have sensitivity and specificity of 100%. Fetal and total DNA are quantified in 22 serum and 12 plasma issued from women with trisomy 21 foetus whom seven were analysed in both serum and plasma.

Results: Cell-free fetal DNA levels is significantly higher only in maternal plasma ($p < 0.001$). The median fetal DNA level in plasma is respectively 41.5 copies/ml and 16.6 copies/ml for Down syndrome group and control group. No statistical difference has been shown in serum or in total DNA in plasma ($p > 0.05$) by using standardized tools and procedure.

Conclusion: We have developed robust calibrators to standardize the step of RQ-PCR analysis and to perform intra and interlaboratory comparison of fetal and total DNA detection or quantification from maternal samples. The stability of these calibrators will permit to develop standardization and quality control programs in a multicentric study and larger applications.

P0479. Evaluation of a new automatic procedure to extract and amplify fetal DNA from maternal serum

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Real-time PCR has become the most widely used amplification system for fetal DNA analysis from maternal serum. However, sensitivity may vary from 31 to 97% depending on the laboratory thus requiring reliable protocols for PCR but also for DNA extraction. Four DNA extraction protocols were evaluated for the detection of fetal RHD gene in 30 sera obtained from RhD-negative pregnant women carrying a RhD-positive fetus; two silica-based column starting with 400 μ l of sample, and a magnetic silica-based automatic system starting either with 400 or 1000 μ l of sample. Quantitative analysis, expressed as crossing-points (Cp), reveals a slightly but significant increase of sensitivity with the automated protocol starting with 1000 μ l of serum when compared with the three others that gave similar results (33.10 vs 34.08 vs 34.12 vs 34.39). A prospective evaluation of this automatic "large volume" procedure was started; 87 sera were tested either for fetal SRY (n=62) or RHD (n=25) gene. Results showed a perfect concordance with the conventional protocol usually performed in our laboratory. Neither false negative nor false positive results were observed. Furthermore, quantitative analysis of the positive sera (n=45) confirmed the slight significant increase of sensitivity with the fully automated procedure with a mean Cp values of 36.40 (vs 37.74 for the conventional one) resulting from the possibility to treat a larger volume of sample. The herein described automatic procedure provides therefore a highly efficient and safe procedure for fetal DNA in maternal serum.

P0480. Mosaic 21-trisomy with varying ratio observed by prenatal screening

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Twenty-one years old gravida was referred for prenatal examination because of cystic fibrosis of her first child (Δ F508 homozygote) on 14th week of pregnancy. DNA examination of chorion biopsy showed that the fetus is a normal homozygote. Screening for chromosomal aneuploidy by interphase FISH gave unexpected result: there were three of 21 specific signals in five of the 50 cells (10 %) examined. In the same sample, 85% of examined metaphase (17/20) 47,XX+21 karyotype were observed. Because of the discrepancy between the interphasic and metaphasic cells examined, repeated sampling was indicated. In the sample from the amniocentesis achieved on the 16th of gestation there were three 21-specific signals in 50% of cells (30/60) examined by interphase FISH, while all of the 11 metaphases (100%) analyzed trisomy 21 were noticed. Although we proposed fetal blood sampling to shed more light on the ratio of mosaicism, the parents following detailed information regarding the results of the previous examination and the presumable prognosis, requested termination of pregnancy. In fetal blood we could complete only interphasic examination, which showed three 21-specific signals in 130 of the 200 cells (65%) examined. In fetal fibroblast cells, we found trisomy 21 in 70% (105/150) and 100% (70 mitosis) examined in interphase and metaphase cells, respectively.

In the course of aneuploidy screening in more than 600 cases, we did not find such a great difference between interphasic and metaphasic cells. We found also interesting the different proportion of mosaicism in samples gained from different dates of the pregnancy.

P0481. Results of first trimester Down syndrome screening in Saint-Petersburg.

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Since 2003 first trimester screening included measurements of nuchal translucency (NT), nasal bone, serum markers (PAPP-A and free beta-HCG) and maternal age. We used ultrasound scanner Aloka SSD-2000. NT measurements was made according to FMF guidelines. Biochemical screening in first trimester was performed with Wallac

equipment (Pribori Oy, Moscow) and risk calculation was made by Life Cycle software. Samples of 1089 patients was investigated. Median level of PAPP-A in Saint-Petersburg was about 35% higher compared recommended one, while level of free β -hCG didn't differ significantly. 380 women (35%) were of 35 years old and elder. High risk of chromosomal diseases was revealed in 29% of this group (cut off 1/250) and 85 % (7 of 8) cases of Down syndrome were detected. There were 4.7% of 709 patients at risk level less than 35. One fetus with translocation variant of DS was detected in young woman (PAPP-A = 0.76 MoM, free β -HCG = 1.45 MoM, NT = 4.77 MoM). In 3 fetuses with Edward's syndrome PAPP-A = 0.22 MoM, free β -HCG = 0.2 MoM. We also detected 1 fetus with triploidy, 1 - with tr16, 2 - 47,XXY and 6 - 45,X.

Combined screening in the first trimester of pregnancy increases the efficacy of prenatal detection of fetal chromosomal diseases.

P0482. PAPP-A and proMBP proteins in contingent prenatal biochemical screening and detection of different types of coronary artery diseases

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PAPP-A/proMBP complex and free- β hCG were evaluated by degree of deviation from 1.6-1.9 MoM. Kryptor system was used for first and second trimester screening (AFP and β hCG), Elipse/LifeCycle software for aneuploidy risk evaluation and software for first and second trimester integrated risk determination. Study of 1825 samples indicates, that category I. (normal values) and II. (border-line values) of PAPP-A/proMBP are without risk of aneuploidies. Category II. indicate increased risk of abnormal prenatal development only. Category III. (abnormal one biomarker) suggests increased risk of autosomal aneuploidy ($p=0.05$). Category IV. (both analyte abnormal levels) and V. (increased β hCG, decreased PAPP-A, increased NT) point out the highest risk of autosomal/X-chromosome aneuploidies ($p=0.001$) with six-times increased risk of preeclampsia, abortions and prematurity. The contingent screening is recommended in categories II.-III., since for categories IV. and V. invasive prenatal diagnosis is indicated, if amniocentesis/placental biopsy are not contraindicated due to high abortion risk. No single case of trisomy 21 or other severe aneuploidy was missed. The amnio-PCR is performed in high risk/late referred pregnancies or to decrease mother's anxiety. PAPP-A/proMBP screening was applied in adult patients with different types of coronary artery diseases (CAD). No increased levels were found in stable CAD. Highly increased levels were disclosed in unstable CAD with highest increase in NSTEMI and STEMI myocardial infarctions with area 0.92 under the ROC curve. PAPP-A/proMBP complex is earlier and more sensitive marker of acute CAD than C-reactive protein, creatine kinase-MB isozyme and cardiac troponin I.

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P0483. Facilitated polar body biopsy by laser-assisted microdissection of the zona pellucida

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Polar body extraction allows to reveal information about the genetic status of the oocyte. This prefertilization diagnosis, which in contrast to preimplantation diagnosis is allowed in Germany, provides an option for couples at high genetic risk. In women of advanced maternal age, polar body extraction is an effective method to eliminate the age-related increased risk of aneuploidies. Genetically altered oocytes may be excluded from in vitro fertilization.

Polar body extraction is a safe and accurate technique. However, it

may be critical for the oocyte as the zona pellucida has to be opened to get access to the polar bodies. The most convenient and gentle method is laser-assisted microdissection. An UV-A laser of 337nm is used to open the zona pellucida in a completely non-contact way. Subsequently a blunt-ending pipette instead of a sharp one can be used for polar body extraction. This procedure may decrease the degeneration rate of oocytes.

P0484. Telomeres lenght differentiation between newborns and their mothers

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Telomeres are repeated DNA sequences that cap and protect ends of chromosomes. Telomere length is known to constitute a largely heritable trait, maintained during fetal development by the enzyme telomerase but shortening with each division in somatic cells after birth. Much attention has focused on the implication of telomere depletion with biological age, especially the association of reduced telomere length for the development of cardiovascular disease. It is against this background that we analyzed 38 pairs of matched blood samples from newborns and their mothers for genome wide telomere length, using real time quantitative PCR. Newborn babies had significantly longer telomeres in comparison to their mothers (mean $9,031 \pm 1,511$ bp vs. $5,960 \pm 1,044$ bp, $P<0.001$). Surprisingly, however, there was no correlation between maternal and newborn telomere length ($r = 0.172$, $P=0.301$), and the range of variation between mothers was small in comparison to that of their babies (range 4,087 - 8,042 bp vs. 6,439 - 12,243 bp). Consistent with previous reports we could not detect any statistically significant correlation with telomere length in this limited maternal age range (20-39 years with majority aged 27-32). Neither did the substantial inter-individual variation between newborns relate to sex or birth weight in this cohort of normal full-term babies. This new information is of special importance as telomere length may constitute a biological marker for predicting individual risk of developing cardiovascular disease later in life - and telomere differentiation may aid identification of fetal cells/DNA in maternal circulation.

P0485. The preimplantation genetic diagnosis in couples with balanced chromosomal rearrangements

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The preimplantation genetic diagnosis (PGD) is an established technique for patients at risk of transmitting a serious genetic disorder to offspring. One of possible indications for the PGD is when couples carry balanced chromosomal rearrangements.

This is a retrospective review of data from a single center. We initiated a process of PGD for 19 cycles after the identification of a parental balanced translocation. We performed PGD for translocation and aneuploidy screening using fluorescent *in situ* hybridization (FISH). Specific FISH protocols were developed for every couple.

One or two blastomeres were aspirated on day 3 after in vitro fertilisation (IVF) and analyzed with FISH technique. We used probes for aneuploidy screening and individual specific probes for chromosomal translocations. A total of 249 embryos were obtained, 228 embryos were suitable for FISH analysis. 36 embryos (15%) were diagnosed as normal or balanced. 175 embryos (76, 75%) were aneuploid or unbalanced for the translocation. 25 (10, 96%) unaffected embryos were transferred in 15 (78, 9%) cycles, achieving 3 pregnancies (implantation rate: 20%).

All antenatal amniocentesis confirmed the initial diagnosis. Post-natal physical examination showed no evidence of major abnormalities. PGD is an alternative method allowing selected couples, with chromosomal abnormalities, to have healthy children. PGD may increase the implantation rate in infertile couples seeking IVF assistance. It has the advantage of avoiding repeated spontaneous abortions or therapeutic termination of pregnancies resulting from abnormal embryos.

P0486. Prenatal diagnosis of a complete parental isodisomy in a live fetus coexisting with partial hydatiform mole

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Objective : The aim of this study is to highlight the outcome of partial hydatidiform mole (PHM) with a complete isodisomic live fetus.

Subject and methods: A 27 years-old Tunisian women; expecting her third child; was investigated by ultrasound, pathological, cytogenetic and molecular techniques.

Results: The ultrasound scan performed at the first trimester identified a hydatidiform mole with admixture of some normal-appearing villi with typical molar villi with a hypotrophic live fetus. The fetus karyotype was cytogenetically normal (46,XX) according to the examination of amniotic fluid cells, but the chromosomes of each pair were extremely identical. But molecular analysis of Amniotic fluid cells DNA found homozygous genetic markers.

Discussion: Homozygous genetic markers are paternal markers. So the fetus is isodisomic for the 23 pairs of chromosomes, transmitted by his father in duplicate.

Conclusion: Prenatal testing for fetal karyotype and genetic markers is therefore essential in deciding continuation and prognosis of the pregnancy. For this reason the termination of the pregnancy was decided.

P0487. Carrier detection and prenatal testing in families with X-linked spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy (SBMA) is a rare late-onset motor neuron disorder characterized by slowly progressive weakness of the proximal muscles of limbs, bulbar symptoms, muscular atrophy, and endocrinological abnormalities (oligospermia, impotence, gynaecomastia and diabetes mellitus). The responsible mutation is a (CAG)n repeat expansion in the first exon of the androgen receptor gene (Xq12). Here we report the study of this trinucleotide repeat in 55 members of 16 Greek families with SBMA by electrophoretic analysis of PCR products containing the polymorphic (CAG)n. The normal range, found in 200 healthy males of Greek origin, was 16-29 repeats (median 21 repeats). An abnormal size of 40-42 repeats was detected in 17 males confirming the clinical diagnosis of SBMA, as well as in 11 female carriers in heterozygosity with a normal allele. Evaluation of the (CAG)n repeat in 36 parent-child pairs showed that 35 transmissions were stable, while in one case there was a contraction of one repeat (from 41 to 40). In four families, twelve prenatal tests were performed using embryonic DNA derived from CVS at 11th-12th weeks of pregnancy. Four prenatal tests resulted in positive finding of mutations in male embryos, and the respective pregnancies were terminated, after genetic counseling. The remaining pregnancies resulted in the birth of six healthy female carriers, one healthy female and one healthy male. The availability of molecular diagnostic testing for SBMA is very useful for clinical purposes (as in differential diagnosis from other motor neuron syndromes) and genetic purposes (carrier detection and prenatal testing).

P0488. Cordocentesis-diagnosed Congenital disorder of glycosylation type 1a (CDG1a) in a fetus with hydrops

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This fetus presented with hydrops fetalis and marked ascites on routine fetal morphology screening ultrasound scan at 19 weeks gestation

. As there was some hyperechoic change in bowel wall suggesting cystic fibrosis, fetal blood was collected in EDTA by cordocentesis. Transferrin isoelectric focussing of this plasma sample showed a distinctly abnormal pattern, consistent with congenital disorder of glycosylation type 1a. The fetus died in utero at 20 weeks gestation. At autopsy, there was severe cutaneous oedema and inverted nipples. Histopathological examination showed cerebellar hypoplasia. Other causes of hydrops were excluded by maternal serum testing for blood group isoimmunisation, TORCH or parvovirus infection and lupus autoantibodies. The delta F 508 cystic fibrosis mutation was not detected. Phosphomannomutase enzyme activity in cultured amniotic fibroblasts was 0.89 nmol/min/mg of protein, intrabatch controls 7.1 and 7.8, affected range (skin fibroblasts) <2.1. Phosphomannoisomerase enzyme activity was normal, confirming the diagnosis of CGD 1a. Mutation analysis is underway. We believe this is the first reported case of abnormal fetal transferrin isoforms, although false negative results in this condition are well recognised. Fetal blood testing has enabled the diagnosis of a congenital disorder of glycosylation type 1a in this fetus.

P0489. Fetal abnormalities in USG as an indication to prenatal diagnosis (PD)

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4095 prenatal cytogenetic tests were performed in the period 1999 - 2003.

In 575 cases (14%) fetal abnormalities detected by USG were the indication to PD. In 115 out of these cases (20%) chromosomal aberrations were found. This constitutes 46% of abnormal karyotypes detected in the overall group. Out of 115 aberrations - 102 were numerical ones: tris 18 - 28, tris 21 - 27, tris 13 - 7, Turner s. - 26, triploidy - 8, unbalanced structural rearrangements were found in 13 cases.

Among USG abnormal findings most frequent were congenital heart disease (CHD) - 139 cases, hydrocephalus - 102 cases, increased nuchal translucency (NT) - 83 cases.

In 68 isolated CHD cases - chromosomal aberrations were found in 15%. In 71 cases with multiple fetal abnormalities, including CHD, chromosome aberrations were found in 50%. No aberrations were found in 55 cases of isolated fetal hydrocephaly. However when hydrocephaly was accompanied by other abnormalities cytogenetic aberrations constituted 27%.

In 56 cases with increased NT as an only marker of aneuploidy - chromosomal aberrations were found in 13%. However karyotype abnormalities were documented in 19 of 27 cases (71%) of increased NT with other fetal abnormalities.

We observe a steady increase of USG indications to PD: in 1999 - 10%, in 2003 - 18%.

P0490. Screening for ΔF508 CFTR and 35delG Connexin26 mutations from maternal dry-blood samples in the 1st trimester of pregnancy

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AIMS Cystic fibrosis has a carrier frequency of about 1/28 to 1/40 among the white Caucasian population and an incidence of about 1 in 3000 live births. One major mutation, ΔF508, accounts for the majority of the observed CF mutations in the Caucasian population. Pre-lingual, non-syndromic autosomal recessive deafness (NSRD) is also a relatively common hereditary disorder, affecting approximately 1 in 2500 newborns. One specific mutation in the CJB2 (Cx26) gene, termed 35delG, has been shown to be present in more than 70% of affected individuals and with a carrier frequency of about 1 in 40. We present our data and evaluation of a routine screening program for these mutations in pregnant women in the 1st trimester of pregnancy, utilizing maternal dry-blood samples.

MATERIALS & METHODS Maternal DNA was rapidly isolated from dry-blood spots and the ΔF508 and 35delG mutations were identified

by PCR analysis using fluorescent primers followed by analysis on an ABI 310 automated sequencer. The results were reported within 48hrs, along with the results of the biochemical screen.

RESULTS The detection method readily and unambiguously identified the ΔF508 mutation in 17 out of the 923 samples analyzed and the 35delG mutation in 2 out of 61 analyzed. This corresponds to a carrier frequency of approximately of 1/55 (1.8%) for ΔF508. The carrier frequency for 35delG is about 1/30 (3.3%), although the sample size is yet too small for accurate determination.

CONCLUSIONS The screening for the ΔF508 CFTR mutation and the identification of women carriers during the 1st trimester of pregnancy has obvious advantages and benefits over other approaches designed to prenatally detect this mutation in the fetus. The early detection of these 2 major mutations may be considered as a wide-scale prenatal screening strategy, affording the timely management of families at risk for CF and/or NRD.

P0491. Comparison of developmental characteristics of implanted and non-implanted advanced blastocysts

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Background: In single embryo transfers (SET) embryo selection is of crucial importance. The present retrospective study aimed to define which embryo parameters might be related to the implantation potential of selected advanced blastocysts.

Materials and Methods: In cycles with SET, developmental characteristics of implanted (group A) and non-implanted (group B) advanced blastocysts were compared. The following developmental parameters were compared between the two groups: number of blastomeres on day 2 and on day 3, fragmentation rate on day 3, compaction on day 4 and cleavage pattern. Statistical analysis was performed by χ^2 square test and *t* test.

Results: From the different parameters analysed, only the fragmentation rate on day 3 was different between groups A and B. Blastocysts with >10-50% fragments on day 3 showed a significantly lower implantation rate (29.7%) than those with ≤10% fragments (49.4% *P*=0.03).

Discussion: Fragmentation rate on day 3 was related to the implantation potential of advanced blastocysts and should be better taken into account in the selection of the best blastocyst for transfer. Since the choice of the blastocyst for transfer was performed on the basis of already established criteria, it may be assumed that the selection on day 5 was carried out properly.

P0492. Loss of expression of MMR proteins; hMLH1, hMSH2 and hPMS2, in secondary and therapy-related AML and MDS patients.

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DNA mismatch repair (MMR) genes are predominantly inherited DNA-repair genes that, when mutated, confer susceptibility to cancer. Our goals were to study the protein expression of these genes in AML and MDS patients. Twenty-eight AML and 7 MDS patients were included in this study. Expression of three MMR proteins (hMLH1, hMSH2, hPMS2), plus the methylation status of promoter region of *hMLH1* and *hMSH2* genes, were investigated in these 35 patients. In addition, a panel of 12 microsatellite markers was selected to look for genetic instability associated with a mutator phenotype (RER+) in these patients. Lack of expression of at least one protein (MMR-) was seen in 11 of 28 AML cases (39.3%), and in 2 of 7 MDS cases (28.6%). This rate was 60% (3/5) in secondary, 50% (3/6) in therapy-related and 29.4% (5/17) in *de novo* AML patients. Both t-MDS patients were MMR-, while all 5 *de novo* MDS patients were MMR+. The rate of MMR- was 61.5% in RER+ AML patients (8 out of 13) while this rate was 20% in RER- AML patients (3 out of 15). All the patients with hypermethylation of either *hMLH1* or *hMSH2* genes showed lack of expression of the

same protein. Our results suggest that abnormalities of DNA mismatch repair due to lack of MMR protein expression is higher in secondary and therapy-related AML and MDS patients than this rate in *de novo* patients. Our data also showed a strong correlation between RER+ phenotype and lack of expression of MMR proteins.

P0493. Molecular cytogenetics of ovarian granulosa cell tumors by comparative genomic hybridization

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Objective. Patients with stage I granulosa cell tumors (GCTs) may occasionally develop metastasis, which is hard to predict using pathologic criteria. It is interesting to elucidate whether certain chromosomal imbalances (CIs), detected by comparative genomic hybridization (CGH), could be useful prognostic markers. **Methods.** CGH was used to identify CI(s) in 37 adult-type GCTs from 36 women. Nonrandom CIs were compared with clinical and pathological features to evaluate their significance as a prognostic marker. **Results.** Twenty-two (61%) of the 36 primary tumors had CIs. One woman's tumor showed identical CIs to another tumor that occurred in contralateral ovary 2 years later, supporting a metastatic nature. The nonrandom CIs included losses of 22q (31%), 1p33-p36 (6%), 16p13.1 (6%), and 16q (6%) and gains of 14 (25%), 12 (14%), and 7p15-p21 (6%). No tumor exhibited high-level amplification. The associations between each CI and pathological features, including the growth pattern, tumor size, and mitotic activity, were not evident. The only CI repeatedly detected in tumors with metastasis was monosomy 22, which presented in 2 of the 4 cases with metastasis but also in 2 of the 5 cases without recurrence for more than 5 years. **Conclusions.** Monosomy 22 was the most common CI in GCTs, which often coexisted with trisomy 14 (in 55% cases). Deletion of 22q seems to be, albeit not very specific, associated with the risk of early metastases of stage I disease. The role of loss-of-function mutation(s) of certain putative tumor suppressor gene(s) on 22q is worthy of further investigations.

P0494. Analysis of *Ile105Val* Polymorphism of GST-P1 gene in esophageal squamous cell carcinoma from Iran - case control study

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To investigate the genetic association between functional polymorphism (*Ile105Val*) in Glutathione-S-transferase (GST-P1) gene and incidence of esophageal squamous cell carcinoma (ESCC), the GST-P1 genotypes were determined by direct DNA sequencing and RFLP analysis in 25 patients with ESCC (Fars ethnic group), 13 patients had *p53* gene mutations in their tumors, and 83 unrelated healthy controls (42 Turks and 41 Fars ethnic groups). The incidence of esophageal cancer among Turks and Fars ethnic populations are recorded to be moderately high and low respectively. The patients carrying the G allele of GST-P1 (AG +GG) had more frequently CpG transition mutation in the *p53* gene in their tumor compared with patients with the AA genotype (*p* value = 0.00029). But the frequency of A allele was demonstrated more among ESCC patients. Polymorphisms in GST-P1 occurred significantly different in Fars and Turks ethnic groups and the G allele of GST-P1 (AG + GG) had more frequent among Turks ethnic group similar to Fars patient group with CpG transition mutation for *p53* gene. Our finding suggested that GST-P1 genotype may play an important role in developing the CpG transition mutation for *p53* gene and the developing esophageal tumor among Fars ESCC patients. This work was supported by NIGEB project number 148, 197 and IDB grant to F.B.

P0495. PIK3CA mutations in familial colorectal and endometrial carcinomas

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Phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is activated in multiple cancers, which is associated with malignant cellular behavior. Activation may result from activating mutations in PI3-kinase genes or inactivating mutations in the tumor suppressor gene PTEN. PI3K/AKT and RAS pathways have been proposed to converge since binding of PI3K to activated RAS may activate the catalytic domain of the PI3-kinase (PIK3CA). While PIK3CA mutations occur with variable frequencies in sporadic tumors, their role among familial tumors is unknown.

We explored the oncogenic role of PIK3CA mutations in colorectal (CRC) and endometrial carcinomas (EC) from patients with hereditary nonpolyposis colorectal cancer (HNPCC) with germline mutations in DNA mismatch repair (MMR) genes, or patients with familial CRC or EC without germline mutations in known genes. We focused on exons 1 (p85 binding), 9 (helical domain), and 20 (kinase), representing known mutational hotspots in CRC.

Somatic PIK3CA mutations were found as detailed in Table 1. PIK3CA mutations, and immunohistochemically detected PTEN inactivation were mutually exclusive. Likewise, mutations in PIK3CA and KRAS were mutually exclusive.

Table 1. Proportion of tumors with PIK3CA mutations according to mismatch repair gene germline mutation status

	MMR gene germline mutation		
	present	absent	total
CRC	4/46 (8.7%)	2/22 (9.1%)	6/68 (8.8%)
EC	10/61 (16.4%)	2/30 (6.7%)	12/91 (13.2%)
total	14/107 (13.1%)	4/52 (7.7%)	

PIK3CA mutations were predominantly associated with EC with MMR gene germline mutations. Mutually exclusive involvement of PIK3CA vs. PTEN or KRAS suggests that alterations of each gene are sufficient to activate the PI3K/AKT signaling pathway in colorectal and endometrial carcinogenesis.

P0496. Relative fluorescent quantitation using capillary electrophoresis for assaying Loss of Heterozygosity in tumor samples

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A variety of capillary electrophoresis based fragment analysis applications require the measurement of peak height comparisons across samples as a relative quantitation method. Some of these applications include screening for Loss of Heterozygosity (LOH), Microsatellite Instability (MSI) and detection of chromosomal deletions and duplications. The success of these assays depends on having optimized chemistries, a robust and reliable electrophoresis platform as well as flexible, accurate analysis software. In this study we use a LOH assay to demonstrate relative quantitation using capillary electrophoresis. Microsatellite markers were run on the Applied Biosystems 3130x/Genetic Analyzer and peak heights were compared across paired samples from tumor and healthy tissues. Using GeneMapper® v3.7 Software candidate LOH samples were flagged for easy identification and review. Our results highlight the 3130 Series Systems in conjunction with GeneMapper® Software as an optimal solution for relative fluorescent quantitation assays.

P0497. Hereditary Non-Polyposis Colorectal Cancer - Where does the buck stop?

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We are auditing the services and quality of care received by Hereditary Non-Polyposis Colorectal Cancer (HNPCC) families under the care of the London Regional Genetics Centres. All HNPCC families with a known mutation, or microsatellite instability or loss of protein on immunohistochemistry testing are included in the audit. Regional Genetic Centre notes are being assessed and follow-up details ascertained from information held by their family doctor. Currently 136 families have been assessed, including 602 at-risk family members of whom 294 are known to live in the geographic area studied. One third of these families do not fit the Amsterdam (Modified) Criteria. 180 individuals have had genetic testing carried out; in 162 individuals tested there was evidence in the notes that counselling was offered and in 162 cases a mutation testing consent form was found. 158 individuals were recommended a surveillance protocol by the genetics centres, but only in 13 cases did the patient notes specify which member of the combined services was responsible for ensuring follow-up. From the available data, 47 of 158 individuals were documented to be adhering to the recommended surveillance protocols. 56 abnormalities from 234 screening episodes were detected, 9 resulting in the detection of a cancer. Further, we have investigated the spectrum of cancers within families, in relation to the specific HNPCC mutation. More colorectal cancer is seen in families with MLH1 mutations and there is a marked increase in urothelial cancers in those with MSH2 mutations.

P0498. Systematic functional testing of the 3p21.3 critical region for tumour suppression of lung cancer

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In lung cancer, a critical region of 370 kb containing 19 well-defined genes has been defined in the 3p21.3 region. In a systematic test for tumour suppressor activity, we have introduced seven PACs overlapping the critical region into a well-transflectable lung cancer cell line. We have used PACs to make it likely that the genes are accompanied by their main regulatory sequences. We check stable transfectants for complete PAC integration, purity, single site of integration and absence of amplification, and have injected clones meeting all criteria into nude mice for tumourigenicity studies. In nine experiments, each on 24 mice, a PAC has been subcutaneously injected on either side of every mouse. Per experiment we used for one side of the mice one of two transfectant clones containing one of the PACs, for the other side of the mouse one of two transfectant clones containing another PAC. The tumours were carefully isolated, measured and weighed, four weeks after injection. The mean weight of the tumours caused by transfectants containing the centromeric PAC was significantly less than that of the tumours caused by transfectants containing one of the other six PACs. This centromeric PAC contains two genes (PL6, 101F6). Our result suggests that one or both of the genes are more important for tumour development than genes on the other PACs.

P0499. The yield of chromosomal aberrations frequency in children with acute leukaemia using methods GTG banding, FISH, CGH, HR-CGH

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We report results from the study of 33 children with acute lymphoid (25) and myeloid (8) leukaemia. Karyotyping of bone marrow cells is an important step for the precise diagnosis and for the choice of the adequate treatment in leukaemic patients. Twenty six patients were

examined in the time of diagnosis and seven in relapse.

The cytogenetic analyses were performed on 24-hours bone marrow cells cultured in RPMI 1640 according to the standard methods. GTG banding technique revealed clonal karyotypic abnormalities in 45% (15) samples.

Cytogenetic findings were confirmed by fluorescence *in situ* hybridization (FISH) method.

Comparative genomic hybridization (CGH) was used on bone marrow samples obtained from this patients in time of diagnosis or relapse. CGH showed new DNA copy number changes in 70% (23) patients. For the first time we were tested the modification of CGH. We show examples of submicroscopic chromosomal imbalances being detected with this high resolution CGH technique (HR-CGH). By HR-CGH we detected new chromosomal aberration in more than 75% cases.

Here we present comparing among GTG-band karyotyping, CGH and HR-CGH results.

P0500. Towards a better definition of unclassified genetic variants in the BRCA1 and BRCA2 genes: the clinical approach.

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One third of the nucleotide variants in BRCA1 and half of those in BRCA2 are genetic variants of uncertain significance, also known as unclassified variants (UVs). Pre-symptomatic testing is not possible and genetic counseling can only be based upon the clinical features and family history.

Whether patients with an UV have different clinical features than those with a mutation in the BRCA1/2 genes had not been investigated. Using BRCAPRO and Myriad II models, we retrospectively obtained the mutation probabilities in 24 patients with an UV. The UVs included: in BRCA1: Arg841Trp, Asp1739Gly, and IVS19: 5313 -25 A>C, and in BRCA2: Tyr42Cys, Ser384Thr, Lys467Arg, Pro655Arg, His1085Arg, Ser1750Phe, Arg2108His, Thr2337Ile, Ala2717Ser, Glu2856Ala, and Lys2950Asn. Secondly, we compared their clinical features and family history with those from 46 patients with an established pathogenic mutation.

The probability to detect a mutation was significantly lower in the group with UVs than in those with mutations (BRCAPRO: $M \pm SD.297 \pm .312$ vs. $.627 \pm .315$ $p=.001$; Myriad II: $M \pm SD.124 \pm .090$ vs. $.283 \pm .176$, $p=.001$). Presence of tumors other than invasive breast and ovarian cancer, number of affected relatives and of tumors among relatives correlated with that difference. The last two variables were independent predictive factors of finding either an UV or a mutation.

The combined probability data show significant differences between both groups. Individual probabilities ("low" vs. "high") can be regarded as a help to guide the clinical management of patients with an UV in those genes. However, with the clinical criteria, evaluation of the pathogenicity of an UV should also include biochemical and epidemiological criteria.

P0501. Loss of 18q as a predictive factor for chemotherapy in stage II colorectal cancer

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Colorectal cancer is the second most common cause of death from cancers in Slovakia. Patients diagnosed with colorectal cancer are divided into clinical stage I - IV according to the level of tumor's invasion.

The most discussing stage, when concerning adjuvant chemotherapy, is stage II characteristic for metastatic negative lymph nodes and other organs. Stage II-patients are not prognostically unique at all. One subgroup shows prognostic similarity to stage III (worse prognosis), the other is similar to stage I. Adjuvant therapy is often not recommended to stage II for its uncertain benefit, whereas patients in stage III with metastasis in lymph nodes undergo chemotherapy as a standard therapy.

Recent studies have shown that what divides patients in stage II into two subgroups might be the status of the long arm of chromosome 18 (18q). If the tumor loses 18q (with potential tumor suppressor gene

DCC) it seems to acquire aggressiveness with high probability of metastatic progression. Therefore loss of 18q is connected with worse prognosis that equals prognosis in stage III. Stage II patients with 18q loss might benefit from adjuvant chemotherapy.

We screened 50 paraffin-embedded (stage II) tumors for the loss of 18q using PCR assay with primers specific for microsatellite markers on chromosome 18q.

Our aims were (1) to assess the effectiveness of chemotherapy, that these 50 patients in stage II had taken, according to their 18q status; (2) to compare PCR-DNA diagnosis with FISH (Fluorescent In Situ Hybridization) focused on marker gene Bcl-2 localized telomeric to DCC.

P0502. The canine model in human genetics

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The canine genome offers a wide field for genetic studies on various areas like e.g. phenotypic diversity, heredity and diseases including cancer. The different canine "purebred" breeding programmes led to expression of various recessively inherited diseases and allowed to observe that the different breeds have particular predispositions for these diseases. The "Canine Genetic Disease Information System" contains the clinical, pathological and genetic features of more than 370 genetic disorders. This offers the rare opportunity for human clinical geneticists to identify breed specific disease associated genes and to study them including their heredity in well documented canine pedigrees. Especially in cases where the number of human patients is small the high number of canine offspring is useful.

In terms of cancer the dog shows additional advantages. The dog enjoys after the human the best medical care of all organisms allowing a detailed surveillance of the cancer, its progression and therapy. At least a dozen distinct canine cancers are hypothesized to be appropriate models for their human counterparts, among those osteosarcomas, mammary carcinomas, oral melanomas, lung carcinomas and malignant non-Hodgkin's lymphomas.

We cloned and characterized more than 10.000 canine ESTs, various disease and cancer related genes and evaluated these genes as targets for development of new therapeutic approaches done in dogs. Among the canine *HMGA1*, *HMGB1*, *ZNF331*, *CCND1* etc. The obtained results show that the canine genes and the deduced proteins are closer to their human counterparts compared to the rodent counterparts emphasising the importance of the canine model for human genetics.

P0503. Insertion of an Alu repeat in exon 3 of BRCA2: a candidate Portuguese founder mutation

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Genetic screening of BRCA1 and BRCA2 in the Portuguese Breast/Ovarian Cancer Families was performed in high risk families. Selection criteria were either 25% of BRCA1/2 combined probability of mutation, according to BRCAPRO or Myriad methods, or male breast cancer in the family.

In 50 non-related families screened for both genes, only one recurrent mutation was found in 3 of them. After PCR amplification of a 425bp product, corresponding to exon 3 of BRCA2 and its intronic boundaries, an extra product with approximately 800 bp was visible in all these 3 samples. The sequencing of this product followed by a BLAST search revealed an Alu motif insertion. Analysis of cDNA was performed using primers in exons 1 and 10 (corresponding to a PCR product of 1.3 Kb) revealing exon 3 skipping. This leads to protein truncation in codon 137 and disruption of the two transcription-activating regions of BRCA2 (residues 18-60 represent a primary activating region; residues 60-105 represent an auxiliary activating region).

Other Breast/Ovarian Cancer Patients were screened for this mutation and we observed it in 5 additional non-related families. Another family had already been studied by another group (E. Teugels, BicDatabase AN: 2971). In two of our 8 families the index patients were male with breast cancer. One of these also developed prostate cancer under 60 as did two of his first degree relatives. Besides this, female breast

cancer was the most frequent cancer observed. Haplotype studies are in progress to confirm if this is a Portuguese BRCA2 founder mutation.

P0504. Co-existence of neocentromeric marker 3q and trisomy 3 in two different tissues in a 10 year old boy having B-cell lymphoma: Support of a gene dosage effect hypothesis

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A very small supernumerary de novo marker chromosome was ascertained during cytogenetic diagnosis of a 10 year old boy with B-cell lymphoma. The marker was C band negative but appeared mitotically stable. The marker was found in 15/15 lymph node cells and 10/14 pleural fluid cells. The marker was duplicated in 5 of 10 pleural fluid cells. An unstimulated bone marrow cultures revealed the presence of trisomy 3 in 10 of 15 cells. Two additional cells had the marker. Fluorescence in situ hybridization (FISH) with chromosome-specific painting probes, alpha satellite probes, subtelomere probes, and physically mapped probes from chromosome 3q was performed and showed the marker to be consisted of inversion duplications of distal portions of chromosome 3q that contain 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 extent of the marker chromosome was characterized by FISH using mapped BAC clone directed onto 3q27 (BCL6) that appeared duplicated. Whole or partial trisomy 3q represents the most recurrent chromosomal abnormality occurring in marginal zone B-cell lymphoma (MZBCL) suggesting that the 3q contains a critical region for the pathogenesis of MZBCL. This case which is unique by the fact that the bone marrow revealed a clone of trisomy 3 whereas the lymph node and pleural fluid showed a neocentromeric marker of 3q supports the hypothesis that a gene dosage effect rather than a specific gene disruption may be involved in the development of this disease.

P0505. The DNA mismatch repair gene *PMS2* is more frequently involved in Hereditary Non Polyposis Colorectal Carcinoma than thought

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PMS2 is a MutL homologue mismatch repair gene, involved in repair of single base mismatches and insertion-deletion loops. To date, seven disease-causing *PMS2* mutations have been reported: three causative for Hereditary Non Polyposis Colorectal Carcinoma (HNPCC) and four causative for Turcot's syndrome. Our aim was to further delineate the role of *PMS2* in HNPCC.

We performed Southern blot analysis in 112 *MLH1*, *MSH2* and *MSH6* mutation negative HNPCC (suspected) families. In 8 patients, selected from 875 familial colorectal carcinoma cases including (suspected) HNPCC cases, on the basis of negative *PMS2* and positive *MLH1*, *MSH2* and *MSH6* staining on a tissue micro array, we performed a mutation scanning by Southern blot analysis (genomic rearrangements) and sequence analysis (point mutations). Subsequently 38 (from the 112) cases were scanned for point mutations. Families with a predicted truncating *PMS2* mutation were further analysed.

Four genomic rearrangements and three *PMS2* protein-truncating point mutations were identified. Three of these seven families fulfil the Amsterdam II criteria. The pattern of inheritance seems to be autosomal dominant with a decreased penetrance (older age of cancer onset) compared to families with pathogenic *MSH2* or *MLH1* mutations. Microsatellite instability and immunohistochemical analysis performed in HNPCC related tumours from proven carriers showed in all an MSI-High phenotype, and absent *PMS2* staining indicative for the involvement of *PMS2* in the aetiology of these tumours.

We show that heterozygous truncating mutations in *PMS2* play a frequent role in HNPCC (suspected) families. We advise mutation scanning in patients with absence of the *PMS2* protein.

P0506. A Protein Phosphatase 2A Regulatory Subunit Can Modulate the Spindle Assembly Checkpoint

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The spindle assembly checkpoint monitors spindle structure in early mitosis, and delays the onset of anaphase until all chromosome-microtubule attachments are appropriate to provide accurate segregation of chromosomes. The Bub1b protein (also known as BubR1) plays a critical role in this mechanism with mutations reported in sporadic aneuploid tumours and in cases of Mosaic Variegated Aneuploidy, an inherited developmental disorder characterised by somatic aneuploidy and tumour predisposition. There is also evidence that Bub1b is involved in attachment sensing and reorganisation, inhibition of the anaphase promoting complex, and initiation of apoptosis in polyploid cells that have escaped mitotic arrest without satisfying the spindle checkpoint. However, the detailed mechanism and regulation of Bub1b activity in these processes is poorly understood. We have identified a specific PP2A regulatory subunit (B56gamma) that interacts with phosphorylated BubR1 in vivo. PP2A regulatory subunits such as B56gamma confer substrate specificity to the catalytic subunit in the PP2A complex. BubR1 becomes transiently phosphorylated in mitosis and we have found that treatment of cells with a PP2A inhibitor extends the period of Bub1b phosphorylation. Furthermore, overexpression of B56gamma in cells reduces phosphorylation of Bub1b and decreases mitotic arrest in response to nocodazole, an agent that activates the checkpoint through spindle disruption. These results suggest Bub1b phosphorylation status is important in mitotic progression and implicate PP2A-B56gamma in its regulation.

P0507. 2300 HNPCC families: research, diagnostics and patient care - The German HNPCC Consortium meets the challenge

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Adequate approaches to HNPCC require clinically and genetically well characterized HNPCC patient cohorts of reasonable size. Since 1999, a registry for HNPCC families has been established in Germany. In a multi-disciplinary approach, six university hospitals collect clinical data of HNPCC families or patients suspected of HNPCC, provide genetic and clinical counseling, tumor tissue analysis, molecular genetic workup, predictive testing and surveillance examinations. Data storage, quality control and biostatistical analyses are performed centrally. A reference pathology center is in charge of histopathology data. So far, 2300 families meeting the Bethesda criteria are included in the study and germline mutations have been identified in 405 families. This cohort, which is one of the largest HNPCC cohorts world-wide, represents an ideal population for analyses in genetics, pathology and phenotype as well as evaluation of diagnostic and therapeutic strategies. Up to now studies on this cohort have produced important results in various fields: Mutation analysis uncovered MLH1,c.1489_1490insC as a frequent founder mutation in the German HNPCC cohort. Studies on genotype-phenotype correlations revealed that MLH1 mutation carriers have a younger age at diagnosis than MSH2 mutation carriers both in regard to first cancer (41 vs. 44 years) and to first CRC (42 vs. 46 years) and that the rate of CRC was higher in MLH1 versus MSH2 mutation carriers. In addition, the German HNPCC Consortium established a novel sequential strategy that can be used to minimize costs of screening diagnostics.

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P0508. Deletion of 9p is significantly more common in metastatic than in primary gastrointestinal stromal tumors

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Objectives: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Current diagnostic criteria could not reliably predict the metastatic behavior of this tumor. Whether presence of certain chromosomal imbalances (CIs) could be applied as reliable genetic markers for such a purpose is worthy to be examined.

Methods: Fifteen metastatic GISTs to the liver, as proved positive for CD117 immunoreactivity, were analyzed using comparative genomic hybridization. The CIs present more often in the metastatic tumors were determined by comparing with the CI pattern of the 28 primary GISTs reported previously (Chen Y et al. J Biomed Sci 2004; 11:65-71).

Results: All tumors had a variable number of CIs, with nonrandom deletions more common than gains. The common CIs were deletions of 15q (80.0%), 1p (73.3%), 9p (53.3%), 14q (53.3%), 22q (46.7%), and 6q (40.0%), and gains of 17q (40.0%). Comparing with the CI pattern of the 28 primary GISTs revealed that the most saline differences were deletions of 9p and 6q. Their detection rates in primary and metastatic GISTs were 7.1% (2/28) and 53.3% (8/15) for 9p deletion, with minimal overlap on 9p13-p22 ($p = 0.001$), and were 7.1% (2/28) and 33.3% (5/15) for 6q deletion, with minimal overlap on 6q11-q16 ($p = 0.040$), respectively.

Conclusion: Among the common nonrandom CIs, 9p deletion was significantly more often identified in the metastatic than in primary GISTs, which appears to be a useful marker to predict the metastatic behavior of this tumor.

P0509. Study of NSCLC gene expression and single nucleotide polymorphism

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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 tumor 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 analyzed gene expression patterns of different NSCLC types using human 30K microarrays in hope to find differentially expressed genes that would help us to predict the survival and clinical course of the patients. In the second step we look for SNPs from the region where the alternatively expressed genes are located. Finally we intend to develop the microarray based analytical test for everyday clinical practice of NSCLC.

P0510. Analysis of VEGF single nucleotide polymorphisms -1154 A/G and 936 C/T in sporadic colon cancer

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Colon cancer is one of the most frequently diagnosed cancers in

Western Societies. Tumor growth requires the formation of new blood vessels, a process called angiogenesis. The most important regulator of angiogenesis is vascular endothelial growth factor (VEGF) that is overexpressed in several tumors. We examined genotype and allele frequencies of two VEGF SNPs, -1154 A/G in the promotor region and 936 C/T in the 3' untranslated region. VEGF -1154 GG genotype is associated with higher VEGF expression, while -1154 AA genotype is associated with lower VEGF expression. Carriers of a 936-T allele have reduced VEGF plasma levels. VEGF low producers genotypes may confer protection, whereas high producers may have a promoting effect on tumor progression. The aim of this study was to determine whether these SNPs might influence the risk for sporadic colon cancer development and progression.

A total 150 colon cancer patients and 150 unrelated cancer-free controls were genotyped for the VEGF -1154 and 936 SNPs using real-time PCR TaqMan® SNP genotyping assay and PCR-RFLP method. Genotype frequencies for VEGF -1154 were 12.6%, 49.6% and 37.6% in control population and 14.2%, 49.6% and 36.2% in colon cancer for AA, AG and GG genotype respectively. Genotype frequencies for VEGF 936 were 69.4%, 29.9% and 0.7% in control population and 69.9%, 26.7% and 3.4% in colon cancer for CC, CT and TT genotype respectively. There were no significant associations between colon cancer susceptibility and -1154 and 936 genotypes however a role of other polymorphisms within VEGF cannot be excluded.

P0511. The relation between telomere length/ telomerase activity and proliferative effects of 190^{BCR-ABL} and 210^{BCR-ABL} fusion genes on hematopoietic cells

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The product of the Philadelphia chromosome translocation, the *BCR/ABL* oncogene, exists in different principal forms that are found in distinct forms of Ph-positive leukemia, suggesting these proteins have different leukomogenic activity. Distinct forms of leukemia are differed from each other with respect to levels of telomerase and telomere lengths also. It has been known that telomeres and telomerase are essential in the regulation of cell life-span and division. It has been hypothesized that the difference in leukomogenic activities of distinct forms of *BCR/ABL* oncogene could be related with the telomere length and telomerase activity of the hematopoietic cells which were transformed by these proteins. We have directly compared the 190^{BCR-ABL} and 210^{BCR-ABL} forms of *BCR/ABL* with regard to the telomerase activity, telomere length and proliferative rate of both myeloid (32D cl3) and lymphoid (Ba/F3) cell lines transformed by both oncogenes. This study tested whether telomerase inhibitors suppress growth of cell lines related with telomere length and telomerase activity. There was found a difference between the telomerase related leukomogenic effects of 190^{BCR-ABL} and of 210^{BCR-ABL}. 210^{BCR-ABL} has been suggested to have a direct effect on telomerase activity in both cell lines.

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P0512. Determination of typical and atypical signals of chromosomes 9 and 22 in CML and ALL cases using by Bcr/Abl ES Dual color probe

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FISH probes with different properties can be used for determination of t(9;22) translocation including; Bcr/Abl Dual color/Single fusion, Dual color/Dual fusion and ES Dual color probes. In this study, we evaluated t(9;22) translocation in 59 cases with ALL, which constitute 42 pediatric and 17 adult cases, and 37 cases with CML by using ES-FISH technique. We observed atypical FISH patterns of chromosomes 9 and 22 in 8 cases of 37 CML cases (22%) and in 10 of 59 ALL cases (17%). These are; trisomy 9 in one Ph- CML (3%) and two ALL (3%)

cases, ASS locus deletion in four cases (11% in Ph+ cases), double fusion of M-Bcr/Abl in two CML (7%) and one ALL cases (2%), four fusion of M-Bcr/Abl in one CML case (3%) and isochromosome 9q in one CML case (3%). Also, we found minor(m) Bcr/Abl fusion in four ALL cases (7%): one case had four copy m-Bcr/Abl signals, and one case had also monosomy 9. Furthermore, three signals for chromosome 22 were found in three ALL cases (5%), one of which had also trisomy 9. Twenty-eight of 37 CML cases (76%) and two of 59 ALL cases (3%) were found to have a simple t(9;22) translocation (M-Bcr/Abl fusion) by ES-FISH.

Given these results, Bcr/Abl ES dual color probe decreases the false-positive results and detects structural and numerical abnormalities on chromosome 9 and 22 simultaneously. Therefore Bcr/Abl ES Dual color probe can be preferable for FISH studies in hematological malignancies.

P0513. Genetic polymorphism analysis of p53 in Iranian population with three different ethnicity and incidence pattern of Esophageal cancer

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The incidence pattern of esophageal cancer is different among Iranian population and is up to 171/100,000. The p53 tumor suppressor gene is involved in the etiology of malignant disease. Several studies were described the polymorphism at codon 72 of the p53 gene (CCC, proline/GCG, arginine) and susceptibility of several types of cancer. Also, it was reported that p53 genotype is involved in patient survival. To investigate the relationship between p53 codon 72 polymorphism and incidence pattern of EC among different ethnicity, we collected samples from healthy population from three different ethnicity groups (Mazandarani from Babol, Turk from Urmieh and Turkomans from Gonbad). Incidences of esophageal cancer in Gonbad, Babol and Urmieh are highest, moderate and low respectively. The p53 Pro72Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and direct DNA sequencing analysis in 126 healthy controls. Among the healthy subjects with Mazandarani, Turk and Turkomans ethnicity, the genotype frequency of p53 Pro72Arg were 15.4% , 36.6% and 39.1 for Arg/Arg, 56.4% , 51.2 and 54.4% for Arg/Pro, 28.2% , 12.2% and 6.5% for Pro/Pro, respectively. Significance difference in p53 allele distribution was observed between Mazandarani and Turkomans healthy individuals ($\chi^2 = 11.15$, $P < 0.01$). In each groups, the distribution of genotypes fits the Hardy-Weinberg equilibrium. Our finding suggested that p53 genotype play an important role in developing and an increasing incidence pattern of esophageal tumor among Turkomans.

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P0514. New complex variants of the simple translocations in leukemia cases.

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t(9;22), t(8;21) and t(1;7) translocations are frequently observed in CML, AML-M3 and ALL respectively and complex variants of these translocations can be observed in leukemia cases rarely. We present here, three cases with new complex variant translocations, First case was a 60 year old male diagnosed as CML. Bcr/Abl fusion gene detected by FISH and RT-PCR. Karyotype of bone marrow cells was 46,XY[10]/46,XY,t(1;6;9;22)(p36.1;p21.3;q34;q11)[10]. Patient treated with Glivec subsequent to INF- γ therapy for two years. He is currently at follow-up as chronic phase CML. Second patient was a 68 years old male diagnosed as AML-M2. Karyotype of bone marrow cells was 45,X,-Y,t(8;16;21)(q22.1;q13;q22)[9]/46,XY, t(8;16;21)(q22.1;q13;q22) [13]. AML/ETO fusion detected by FISH. Patient treated with Ara-C + Ida (7+3) as remission induction and two courses of HIDAC as consolidation therapy. Patient achieved remission but died because of the early relapse. Third case was a 16 years old male diagnosed as CML. Bcr/Abl fusion was detected by FISH. Patient treated with Glivec subsequent to INF- γ + AraC for one year. Transformation to ALL

was observed one year later. Karyotype of bone marrow cells at this time was 45,XY, der (7) t(1;7;22)(p31;p21;q13.2),-9[25]. Three courses of the HyperCVAD+Glivec was applied to patient. After given FLAG therapy, he died because of the progressive disease. Our results show that possible alterations of genes located in these breakpoints involved in complex variant translocations might effect relaps and overall survival durations of the patients. Therefore, determination of complex variant translocations at diagnosis might have prognostic significance in hematological malignancies.

P0515. Identification of polymorphisms in exon 4 of the p53 gene among Iranian esophageal squamous cell carcinoma

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It is recorded that most cancers carry *p53* gene mutations. The *p53* protein is considered a key combination in countering stress messages such as DNA damage. The frequency of esophageal squamous cell carcinoma (ESCC), the predominant cancer in esophagus, is very high in northern Iran and identification of *p53* gene mutation is very important to find the major causes of this type of cancer in Iran.

In this investigation, we assessed allele frequency of *p53* polymorphism among ESCC patients with Fars ethnic group. The *p53* Pro72Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and direct DNA sequencing analysis in 166 healthy controls and 35 patients. Among the healthy and ESCC patients subjects with Fars ethnicity, the genotype frequency of *p53* Pro72Arg were 34.3% and 31% for Arg/Arg, 44.6% and 49% for Arg/Pro, 21.1% and 20% for Pro/Pro, respectively ($\chi^2=0.17$, $P>0.9$). No significance differences were found for *p53* genotype distribution among patients and healthy individuals. Our finding suggested that *p53* genotype does not play an important role in developing esophageal tumor among Fars ESCC patients. This work was supported by NIGEB project number 197, 206 and the IDB grant to F.B.

P0516. Assessing NAD(P)H: quinine oxidoreductase $^{609}\text{C}\rightarrow\text{T}$ polymorphism using simple PCR method on formalin-fixed paraffin-embedded human colon and esophageal tumor tissues from Iranian cancer patients

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The NAD(P)H: quinine oxidoreductase (*NQO1*) is applied in the detoxification of numerous endogenous and foreign compounds. It has been shown that homozygous patients having TT allele exhibit negligible *NQO1* enzyme activity. The lack of *NQO1* activity might increase the risk of certain types of toxicity and cancer. We assessed *NQO1* $^{609}\text{C}\rightarrow\text{T}$ polymorphism in genomic DNA isolated from blood and formalin-fixed, paraffin-embedded human colon and esophageal tumor tissues from Iranian individuals by a simple PCR method. Also, the results were confirmed by Direct DNA sequencing. Because the frequency of *NQO1* C609T polymorphism has not been reported among the Iranian population so far. Therefore, the current study presents a simple and feasible method for detection of *NQO1* genotype in Iran. This work was supported by NIGEB projects number 176 and 197.

P0517. Mechanisms of apoptotic induction and cell cycle inhibition in cancer cells by novel compounds synthesized by using analogue synthesis and structure-based drug design

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In recent years, it is known that normal cellular proliferation is tightly regulated by the activation and deactivation of a series of proteins that constitute the cell cycle machinery. The expression and activity of these cell cycle components can be altered during the development

of a variety of diseases, such as cancer. Apart from yielding a new source of therapeutic target, it is likely that manipulating the activity of such proteins will provide an important route for treating cancers. The Cyclin-dependent kinases (CDKs) are Serine/Threonine protein kinases, which play a pivotal role in the transition from the G1 to S, and G2 to M phase of the cell cycle. Therefore, in an attempt to search for a specific CDK inhibitor with minimal side effects, the current authors synthesized an analogue of Toyocamycin and Sangivamycin, MCS-C2. Furthermore, in the course of screening for a novel inhibitor of CDK2 using the Structure-Based Drug Design (SBDD), we isolated CR2-R29 from the chemical library. The present study investigated the selective anti-neoplastic potential with significant low toxicity and mode of action of apoptotic inductions by MCS-C2 in human cancer cells (HL-60, LNCaP, PC-3 and DU145), and CR2-R29 in HeLa and PA-1 cancer cells. The two compounds showed the inhibitory activity to the cell cycle progression, and apoptotic inductions in various human cancer cells, but no effect in normal cells. As the result, we suggest that MCS-C2 and CR2-R29 are novel potent anti-cancer drug candidate through the cell cycle regulation inhibition and apoptosis as CDK inhibitor.

P0518. BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases.

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Studies of breast cancer in Northern Africa have found striking differences with European breast cancer patterns. The size and grade of breast tumors in the Maghreb are increased, while the median age of onset is much younger. The increased size and grade may be explained by late discovery. Differences in diet and lifestyle, particularly earlier and more frequent childbirth, may change breast cancer incidence, as might genetic differences, or combinations of these factors.

We tested the contribution of mutations in *BRCA1* to breast cancer in Algeria. We used the same criteria to define 'familial' cases that we use for our French families, and added early onset 'sporadic' cases suggestive of a genetic predisposition component. All exons and splice-junctions of *BRCA1* were sequenced. QMPSF was used to detect deletions and duplications.

Cases were chosen at the Pierre and Marie Curie Hospital in Algiers according to the following criteria: age of onset before 38 years, or two or more affected first degree relatives. 48 early onset sporadic cases (average age at diagnosis 31.1, range 18 to 38) and 5 familial cases (average age at diagnosis 39.3, range 32 to 53) were included.

We observed six deletions of one to 29 nucleotides, and a change in the donor splice site of exon 5. No nonsense or missense mutations were observed. There was no difference in the average age of mutated sporadic cases (32, range 26 - 37) versus non-mutated sporadic cases (31, range 18 - 38) or mutated familial cases (37.2, range 32-41).

P0519. Molecular study on frequency of single nucleotide polymorphism of DNMT1 among Iranian patients with gastric cancer

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Introduction: Mutations of the *DNMT1* gene have been frequently reported in gastric cancer. Single nucleotide polymorphisms (SNPs) have been presumed to be associated with the genetic susceptibility to cancer. We have hypothesized that SNPs within the *DNMT1* may be associated with sporadic gastric cancer. **Method:** we designed a case-control study of age, sex and ethnicity matched patients with sporadic gastric cancer and healthy controls. Up to now we have enrolled 70 cases and 65 controls. Genomic DNA was extracted and the locus for the SNP: 721186 on exon 20 of the *DNMT1* gene was amplified. We used RFLP reaction by the Acyl enzyme. The undigested amplicons were of 300 base pairs (bp). In case of homozygous state for the CC allele the amplicons were digested to 100 and 200 segments. The heterozygous had three bands at 300, 200 and 100 bp. **Results:** The frequency of the CC, CT and TT genotypes among the cases and controls were: 98% vs. 98%, 0% vs. 0%, 2% vs. 2% respectively. The C and the T allele frequencies among the cases and controls were

98% vs. 98% and 2% vs. 2% respectively. There was no significant difference between the cases and controls. Conclusion: Our result shows that the SNP: 721186 polymorphism of the DNMT1 gene has no apparent association with gastric cancer among a sample of Iranian patients. Further investigation involving more patients and more polymorphisms are required to delineate the true role of DNMT1 polymorphisms in the pathogenesis of gastric cancer.

P0520. Gene expression analysis of PAX2 and PAX8 in patients with sporadic renal cell carcinoma (RCC)

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Background: Sporadic renal cell carcinoma is one of the most common urological malignancies in adults (85%). According to the IARC (The International Agency of Research on Cancer) is Czech Republic on the first world position at incidence and mortality for RCC. The prognosis of RCC is very poor because of high mortality up to 40% and unpredictable progression after tumor abstraction. More precise molecular prognostic markers are required. Genes PAX2 and PAX8 control cell division during embryonic development and play crucial role in tumor development because of stimulation of cell proliferation or/and inhibition of apoptotic program.

Material and Method: Our RCC sample collection (collected since 2002) contains 61 patients. mRNA was isolated from tumor cells and nonmalignant renal cells and converted into cDNA. Expression of PAX genes was analyzed by using relative quantification real time PCR with TaqMan labeled probe. Gene GAPDH was chosen as an endogenous control.

Results: Expression of PAX2 gene was found in 94% and expression of PAX8 gene was found in 89% of analyzed samples. The level of expression of both PAX genes was very variable with the range from hundred times lower to forty times higher compare to the expression of chosen endogenous control. Correlation between level of PAX2 expression and tumor size was found ($p=0.07$) on the cut-off of statistic significance.

Summary: These results haven't got prognostic value yet because of short duration of patient observation. Follow-up clinical data are essential for completion of our research.

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P0521. Differential methylation of LAMC3, SEMA6B, VCIP135 and BIN1 CpG islands in breast cancer identified by methylation-sensitive restriction fingerprinting (MSRF).

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MSRF is designed to screen for altered methylation patterns in genomic DNA. By use of our MSRF modification we have identified four abnormally methylated CpG islands belonging to LAMC3, SEMA6B, VCIP135 and BIN1 genes encoding laminin gamma 3 chain; semaphorin 6B; valosin-containing protein p97/p47 complex-interacting protein p135 and bridging integrator 1, respectively, in breast cancer (BC) DNA samples.

Laminins are major glycoproteins of the basal laminae. By methylation-sensitive PCR we detected methylation of LAMC3 5'-CpG island in 13/54 (24%) samples. We presume that LAMC3 gene abnormal methylation in cancer samples may result in disruption of cellular-lamina contacts resulting in cell cycling disbalance and metastatic spreading.

Several members of semaphorin family have already been shown to take part in cell proliferation and differentiation, cell signaling and interactions with proto-oncogenes. Abnormally methylated CpG island of SEMA6B was detected in 17/54 (32.5%) of BC samples.

VCIP135 gene product containing an OTU (Drosophila ovarian tumor)

domain is known to be necessary for VCP-mediated reassembly of Golgi stacks after mitosis and its possible roles in carcinogenesis are yet to be evaluated. We observed methylation of promotor region VCIP135 in 9/54 (16.7%) samples.

BIN1 encodes a MYC-interacting protein with features of a tumor suppressor. Abnormally methylated CpG island of BIN1 was detected in 11/54 (20%) of BC samples.

We detected no methylation of all investigated CpG islands in control peripheral blood lymphocytes and apparently intact breast tissues. Epigenetic alterations in above genes have not been reported previously.

P0522. Urothelial carcinoma detection with fluorescence *in situ* hybridization

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Cystoscopy and cytology are techniques, which have high specificity but poor sensitivity for urothelial carcinoma detection. Identification of genetic alterations at chromosomal or DNA level in urological malignancies is very important for evaluation of the response against therapy. We have performed FISH analysis by using centromeric and locus specific DNA probes to interphase tumor cells obtained from urine, without prior knowledge of clinical findings, that is pathology, cystoscopy and cytology results. A mixture of fluorescent labeled probes for centromeres of chromosomes 3, 7 and 17 and band 9p21 (p16/CDKN2A) gene was used to assess urinary cells for chromosomal abnormalities, indicative of malignancy. A total of 16 urine specimens from 11 male and 5 female bladder cancer patients were analyzed. Two of these specimens were grade 1, five were grade 2, seven were grade 3 and two of them were grade 2 or grade 3 with pT2a stage. We found three specimens were tetraploid for chromosomes 3, 7 and 17, 3 specimens were tetraploid for these chromosomes and deleted for 9p21, one was triploid for chromosomes 3, 7 and 17 and normal for 9p21, two were triploid for chromosomes 3, 7 and 17 and deleted for 9p21, two specimens were deleted for 9p21, one specimen was normal and we have detected 4 non-specific amplifications. Our results strikingly showed that, these chromosomal pathologies could be detected in not only grade 3 specimens also found in grade 1 tumors, therefore FISH is very sensitive to detect urothelial carcinoma, even in earlier stages.

P0523. Investigation of mitochondrial common DNA deletion (5 Kb) in Iranian patients with Gastric cancer

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Human mtDNA is a supercoil, double stranded circular molecule compromising 16,569 base pairs. It codes for 13 proteins (all subunits of the respiratory chain complexes), 22 tRNAs and 2rRNAs. It has been known that mtDNA is far more vulnerable to mutations than nuclear DNA due to its lack of histone protection, limited repair capacity, and closed to the electron transport chain, which constantly generates peroxide radicals. In solid tumors, elevated expression of mtDNA - encoded subunits of the mitochondrial electron respiratory chain may reflect mitochondrial adoption to gain cellular energy requirements. Mt DNA mutations occur in a wide variety of cancers and may have utility in detection of cancer. We investigated the presence of mtDNA common deletion (a 4977bp deletion which characteristically flanks by direct repeats) using conventional molecular techniques such as PCR, Southern blotting etc. We analyzed DNA from 30 paraffin embedded gastric adenocarcinoma tissues and 30 blood specimens for this deletion from the cancer patients. Our results showed that 40% blood tissues and 26% paraffin embedded gastric adenocarcinoma had ~5kb deletion. Common deletion rate reported in aged people is only 6%, while the percentage of deletion in our specimens was about 10%. It has been known that ATP requirement is increased during cancer development. This may be one of the reasons that we detected more copies of mtDNA with common deletion in our patients. Since

several different mtDNA mutations have been detected in cancers, more investigation needed to reveal the role of mitochondria in Iranian cancer patients.

P0524. Association of two mutations in the *CHEK2* gene with breast cancer

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The 1100delC mutation of the cell cycle checkpoint kinase 2 (*CHEK2*) gene confers an increased risk for breast cancer, but the clinical impact of other *CHEK2* gene variants remains controversial. We determined the frequency of two functionally relevant *CHEK2* gene mutations, I157T and IVS2+1G>A, in two large series of breast cancer cases and controls from two independent populations. Our first series consisted of a hospital-based cohort of 996 German breast cancer cases and 486 population controls, and the second series consisted of 424 breast cancer patients and 307 population controls from the Republic of Belarus. The missense substitution I157T was identified in 22/996 cases (2.2%) versus 3/486 controls (0.6%; OR=3.6, 95%CI 1.1-12.2, p=0.044) in the German population, and in 24/424 cases (5.7%) versus 4/307 controls (1.3%; OR= 4.5, 95%CI 1.6-13.2, p=0.005) in the Byelorussian cohorts. The splicing mutation IVS2+1G>A was infrequent in both populations and was observed in 3/996 German and 4/424 Byelorussian patients (0.3% and 0.9%, respectively) and in 1/486 German control individual (0.2%; adjusted OR=4.0, 95%CI 0.5-30.8, p=0.273). Heterozygous *CHEK2* mutation carriers tended to be diagnosed at an earlier age in both populations, but these differences did not reach statistical significance. Family history of breast cancer did not differ between carriers and non-carriers. Our data indicate that the I157T allele, and possibly the IVS2+1G>A allele, of the *CHEK2* gene contribute to inherited breast cancer susceptibility.

P0525. Genetic and epigenetic pathology of a number of cancer related genes in retinoblastoma.

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Complex testing for structural and functional inactivation of the *RB1* gene in 60 retinoblastomas have revealed a molecular defect in at least one allele in 90% tumors. SSCP and heteroduplex analyses, along with direct sequencing, revealed 47 mutations in *RB1* gene. Loss of heterozygosity (*RB1nt2*, *RB1nt20*, *D13S262*, *D13S284*) of at least one of markers was found in 70% of cases. *RB1* aberrant methylation was detected in 25% analyzed tumors. To investigate the role of methylation-demethylation and its association with retinoblastoma we studied methylation status of *p16/CDKN2A* and *RB1CC1* promoter regions as these genes function as upstream regulators of pRB activity; and promoter regions of *p15/CDKN2B*, *p14/ARF*, *N33*, *MGMT*, *HIC*, *ERα*, *CALCA*, *CDH1/E-cadherin* and *IGF2* tumor related genes by multiplex methylation-sensitive PCR. High methylation frequencies were observed for *CDH1* (60%), *HIC1* (47%), *p16* (15%), and *p14* (22%). Low levels of methylation were detected for *MGMT* (2%), *ERα* (7%), *CALCA* (3%) and *RBCC1* (3%) genes. No methylation was shown for *p15* and *N33* promoter regions. We also detected a loss of *IGF2* gene imprinting in 28% retinoblastomas. The methylation profile was compared for unilateral and bilateral retinoblastomas and for tumors differing in the mechanism of *RB1* inactivation. The mean methylation index in retinoblastomas with combined (structural and functional) inactivation of *RB1* was significantly higher than in tumors with incompletely inactivated *RB1* (P = 0.01). The methylation frequencies of the above genes did not significantly differ between the groups of tumors. These data confirm necessity of methylation profile studying in different tumor types.

P0526. Dependence of Fas and FasL expression on genetic rearrangements in tumor cells

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The development and progression of tumors depends on their interaction with the immune system of the organism and the instability of the tumor cell genome. The aim of this study was to reveal the effect of Fas-dependant apoptosis in tumor cells and splenocytes on genetic rearrangement in tumor cells.

The study was carried out on the mouse hepatoma cell line MH-22a and the sarcoma J-774.

Genetic variability in these tumors and their clonal lines was examined in vitro and in vivo at their proliferation in the subcutaneous connective tissue (SCT) and in the eye anterior chamber (EAC) by RAPD-PCR. Interinduction of apoptosis between tumor cells and splenocytes was carried out at their combined cultivation in vitro during 18 h. Apoptosis was revealed by electrophoresis of low molecular fractions of DNA, by clonogenic survival and flow cytometric analysis. Expression of Fas-receptor (Fas) and Fas-ligand (FasL) was revealed by RT-PCR.

The level of genetic variability in clonal lines of hepatoma MH-22a and sarcoma J-774 varied greatly both in vitro and in vivo. Apoptosis was revealed in tumor cells as well as in splenocytes. Clonal lines of tumor hepatocytes MH-22a and histiocytes J-774 showed heterogeneity in the intensity of Fas and FasL expression. All clonal lines examined showed expression of Fas and FasL, but the levels varied widely. In many cases changes in the fingerprints appeared, coinciding with significant increases of Fas and FasL expression in hepatoma and histiocytoma clones.

P0527. Molecular study on frequency of single nucleotide polymorphism of *TP53* among Iranian patients with gastric cancer

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Introduction: Single nucleotide polymorphisms (SNPs) of *P53* gene have been presumed to be associated with the genetic susceptibility to gastric cancer. We have hypothesized that SNPs within the *TP53* may be associated with sporadic gastric cancer. **Method:** we designed a case-control study of age, sex and ethnicity matched patients with sporadic gastric cancer and healthy controls. Up to now we have enrolled 81 cases and 49 controls. We used PCR-RFLP to distinguish between the alleles at SNP 1042522 and the SNP 1800371. Both loci were amplified by a single PCR reaction which resulted in 240 bp amplicons. For the SNP 1042522, homozygous GG cases were digested to 100 and 140 fragments. The heterozygous cases had three bands at 240, 140 and 100 bp. For the SNP 1800371 homozygous cases for the TT allele were digested to 25 and 215 segments. The heterozygous cases had three bands at 240, 215 and 25 bp. **Results:** The frequency of CC, CG and GG genotypes among the cases and controls for the SNP 1042522 were as follows: 22.7% vs. 14.6%, 77.3% vs. 85.4%, 0% vs. 0% respectively. For the SNP 1800371 all the genotypes were CC among the cases and controls. No significant difference was observed between the cases and controls for either of the SNPs. **Conclusion:** Our preliminary result shows that the SNPs: 1042522 and 1800371 in the *TP53* gene has no apparent association with gastric cancer among a sample of Iranian patients.

P0528. SET (TAF-IB) gene is highly expressed in acute lymphoblastic leukemia patients

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SET gene (TAF1beta) was originally identified as a component of the SET-CAN fusion gene in a patient with acute undifferentiated leukemia (AUL). SET gene encodes a nuclear phosphoprotein that ubiquitously expressed. SET was shown to be an inhibitor of protein phosphatase 2A which involved in regulating cell proliferation and differentiation. SET is also a subunit of INHAT complex. Binding of INHAT complex to histones prevent their acetylation which cause transcriptional repression.

Overexpression of SET can inhibit demethylation of DNA which results in gene silencing. We have hypothesized that overexpression of SET gene may play an important role in leukemogenesis. To test this hypothesis we investigated SET gene expression in bone marrow sample of 57 acute lymphoblastic leukemia patients and 5 control samples from healthy volunteers using quantitative real-time PCR technique. SET gene expression in patients with acute lymphoblastic leukemia was statistically higher than control samples ($p=0.005$). Using Pearson's chi-square test, no significant association between SET gene expression and peripheral WBC count, sex, FAB group and immunophenotype and presence of TEL-AML1 fusion gene in ALL was detected (Table 1). Overall survival and relapse-free survival was not significantly different between high and low SET expressed patients with ALL (n=48, log-rank=0.06, $P=0.80$ n=42, log-rank=0.12, $P=0.71$ respectively). Finally, high level of SET mRNA expression were found in acute leukemia. SET gene may play important role in leukemogenesis.

Clinical characteristics at the time of diagnosis of patients studied				
		Low Expression n	High Expression n	P value (X ²)
Gender (n=57)	Female Male	4 8	17 28	1.000
Age (Years) (n=57)	9 years	0 9 3	1 35 9	0.823
WBC (/ml) (n=55)	100.000/ μ l	6 2 4	33 4 6	0.187
Immunophenotype (n=57)	Precursor B cell T cell	5 7	22 23	0.751
FAB (n=48)	L1 L2	6 5	23 14	0.132
TEL-AML1 (n=55)	Positive Negative	1 10	11 33	0.240

P0529. VHL mutation analysis in sporadic haemangioblastoma

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Von Hippel-Lindau (VHL) disease is estimated to account for ~30% of CNS haemangioblastomas. A clinical diagnosis of VHL disease may be made in patients with multiple CNS haemangioblastomas or a single CNS haemangioblastoma and a family history of VHL disease or an extra-CNS VHL tumour (retinal angioma, renal cell carcinoma or phaeochromocytoma). Previously we identified a germline VHL gene mutation in 4% of apparently sporadic haemangioblastoma cases. Although specific VHL missense mutations may be associated with a phaeochromocytoma only phenotype evidence for low penetrance 'haemangioblastoma only' VHL mutations has not been reported.

We now report a survey of VHL mutations in a cohort of 168 apparently sporadic UK CNS haemangioblastoma patients (60 reported previously). We identified six patients (mean age 34.3 years) with germline VHL gene mutations (two reported previously). Three patients had loss of function mutations (one frameshift, two germline deletions) reported previously in VHL disease and three patients had germline missense mutations (two R200W and one N179D). R200W has been reported previously in patients with VHL disease and cerebellar haemangioblastoma but, when homozygous, causes Chuvash polycythaemia. N179D has not been previously reported.

These preliminary results suggest that (a) all patients with apparently sporadic CNS haemangioblastoma should undergo molecular genetic testing for VHL disease by direct sequencing and MLPA and (b) germline VHL mutations detected in patients with sporadic CNS haemangioblastoma do not necessarily predict classical VHL disease in the proband and carrier relatives. Thus in such cases the family should be counselled carefully.

P0530. Combination of various molecular cytogenetic techniques in detection of structural rearrangements of chromosome 7 in hematological malignancies

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Deletion of long arms of chromosome 7 is one of the frequent chromosomal aberrations in myeloid disorders such as myelodysplastic syndrome (MDS) and acute myeloblastic leukemia (AML), and is associated with aggressive disease and poor prognosis. Both terminal and interstitial deletions of 7q have been reported with variable breakpoints among patients. Only few studies have focused on the analysis of translocations, either balanced or unbalanced, involving deleted chromosome 7q. In our study we used combination of multicolor fluorescence in situ hybridization (mFISH), multicolor banding (mBAND) for chromosome 7 and FISH with locus specific probes for 7q22, 7q31 and 7q35 regions to detect the deletions and rearrangements of chromosome 7. Using classical cytogenetic techniques we examined 31 patients with different hematological malignancies, who had in malignant bone marrow cells 7q deletion or complex rearrangements of chromosome 7. FISH with locus specific probes confirmed the deletion of 7q in 18 patients. The extent of deletion varied, del (7)(q22q35) was the most frequent one (5 patients). Different complex rearrangements of chromosome 7 were proved using multicolor FISH (in 27 patients) and multicolor banding technique for chromosome 7 (in 9 patients). Origin of these rearrangements and their prognostic significance will be discussed in the poster.

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P0531. Genetic detection and clinical significance of occult lymph node metastasis in patients with liver metastasis from colorectal cancer

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Liver transplantation for nonresectable liver metastasis from colorectal cancer has been abandoned in 1994 on account of high recurrence rates and limited long-term survival. The aim of this study was to investigate whether the genetic detection of micrometastasis in histologically negative lymph nodes from colon cancer can be applied to select patients for liver transplantation. We retrospectively analyzed 21 patients with colorectal cancer and subsequent liver metastasis who had undergone a liver transplant within the 1983-1994 interval. Direct DNA sequencing was used to screen tumor material for p53 and K-ras mutations. Eleven of these patients had shown no histologically detectable lymph node metastasis at the time of surgery (pN0), 10 patients with lymph node metastasis (pN1) served as control group. We used mutant allele-specific amplification (MASA) to identify corresponding genetic alterations in regional lymph nodes from colorectal cancer. p53 and K-ras mutations were detected in 12 (57%) and 3 (14%) of 21 patients in the primary colorectal cancer, respectively, and could be confirmed in the corresponding liver metastasis. Nine of eleven histologically lymph node-negative patients were evaluable due to presence of p53 mutation. MASA revealed 6/9 patients to be genetically positive for micrometastasis. Three patients were genetically and histologically negative. These 3 patients showed a significantly longer overall survival ($p=0.011$) of 4, 5 and 20 years, respectively. We conclude that the genetic detection of micrometastasis by p53 and K-ras MASA could be a powerful prognostic indicator to select patients with colorectal liver metastasis who benefit from liver transplantation.

P0532. Mutation analysis of the MYH gene in Czech FAP patients

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Familial adenomatous polyposis (FAP) is inherited predisposition to colorectal cancers characterized by adenomatous polyps in the colon and rectum. The autosomal dominant FAP is associated with germline mutations in the *APC* (adenomatous polyposis coli) tumor-suppressor gene. Germline mutations in the *MYH* gene have been associated with recessive inheritance of multiple colorectal adenomas. 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 individuals with multiple (3 to 100) colorectal adenomas and in *APC*-mutation-negative probands with classic familial adenomatous polyposis (>100 adenomas). Mutation screening involves the entire *MYH* coding region and adjacent intronic sequences. We established mutation analysis using denaturing high performance liquid chromatography (DHPLC) on WAVE system. Deviations were confirmed by direct DNA sequencing.

We have detected compound heterozygotes for two of the most common germline mutations c.494A>G (p.Y165C); c.1145G>A (p.G382D) and several other sequence changes (mutation/polymorphism) either in *MYH* coding sequences or in intronic sequences. Further elucidation of functional role of the altered residues, together with the analysis of other family members, will help to establish a more definitive correlation of these changes with pathogenicity.

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P0533. HER2/neu gene amplification in muscle invasive bladder cancer; FISH analysis on paraffin-embedded specimens

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Studies in bladder cancer have revealed a discrepancy between gene amplification and protein expression of Her2/neu gene and the clinical importance as a prognostic value. Paraffin-embedded specimens from a total of 34 cases which none of them had received radiotherapy or chemotherapy of diagnosed bladder cancer were analysed using dual-color fluorescence *in situ* hybridization (FISH). Dual color hybridization signals belong to chromosome 17/HER2/neu gene and chromosome 17/chromosome 8 were counted within 200 interphase nuclei per specimen. Among the 34 bladder tumor patients 5 cases had HER2/neu amplification with the different copy number ratio and 17 cases had increased copy number for chromosomes 8 and 17. The average of overall survival for the 2 of 5 cases which had HER2/neu amplification less than 5 copy number was 19.3 months and for the remaining 3 cases which had HER2/neu amplification more than 10 copy number was 34 months. Our preliminary results suggest that HER2/neu gene amplifications may play important role in the progression of bladder cancers. Furthermore, over expression of HER2/neu protein without DNA amplification may be dependent on increased copy number of chromosome 17 or polyploidy.

P0534. Polymorphic tandem repeats of three sexual hormone receptors genes in breast cancer

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Case-control studies have reported inconsistent results concerning the association between polymorphisms in the androgen and estrogen receptor genes and breast cancer. While several studies investigated the association between the androgen receptor gene CAG repeat and breast cancer, for the CA and TA repeats in the estrogen receptor genes there are considerably fewer studies.

We have investigated the potential link between three tandem repeats

(CAG, TA and CA) in the androgen receptor, estrogen receptors α and β genes respectively and breast cancer. DNA was isolated from 153 invasive breast tumors and 318 controls, and the three tandem repeats were sized by polyacrylamide electrophoresis. Number of repeats in each allele and the total repeats of both alleles were taken as variables for classification into dichotomous groups using the median of each variable in the control group as cut-off point. Relationship between polymorphic tandem repeats and breast cancer was assessed by multivariate logistic regression models.

The overall success of the model in predicting breast cancer was 74.1% (for a 0.5 cut value). The results suggest that three variables combined, longer CAGsum (≥ 28 ; $p < 0.001$; $OR = 9.262$; 95%CI=3.583-23.945), shorter TA (< 23 ; $p < 0.001$; $OR = 4.195$; 95%CI=1.912-9.206) and shorter CA (< 23 ; $p = 0.028$; $OR = 3.180$; 95%CI=1.131-8.943) repeats could constitute a possible genetic profile associated with breast cancer.

Our data confirm previous reports regarding an association between longer CAG repeats and breast cancer. In addition to that, we found that the combination of long CAG, short TA and short CA repeats are strongly associated with breast cancer.

P0535. The occurrence of a childhood choroid plexus carcinoma should be added to the indication criteria for testing for germline TP53 mutations

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We present five new families of paediatric patients suffering from choroid plexus carcinoma in which we found germline TP53 mutations. Only one of the families conformed to the criteria of the Li-Fraumeni syndrome, and only three (including the Li-Fraumeni syndrome family) met the Chompret criteria for germline TP53 mutation testing. There was no family history of cancer in the remaining two families and/or the parents of the patient were shown not to carry the mutation. Our results give further strong support to the notion that the occurrence of this rare paediatric tumour, especially in combination with a positive family history of cancer, but possibly also without any family history, may be a strong indicator of a germline TP53 mutation. The identification of these five new families prompted us to analyse the efficiency of various clinical criteria for germline TP53 mutation testing. In total, we tested members of 102 cancer families, and found 14 germline TP53 mutations. All but two mutations were identified in families meeting at least one of the criteria (Li-Fraumeni syndrome, Li-Fraumeni-like syndrome (Manchester), or the Chompret criteria). The remaining two mutations were identified in two families with paediatric choroid plexus carcinoma patients mentioned above. Furthermore, in each of the total of six families tested with occurrence of this tumour a TP53 mutation was found. We suggest therefore that the occurrence of this tumour is added to the indication criteria for germline TP53 mutation testing. Supported by grant MSM0021620813 from the Ministry of Education of the Czech Republic.

P0536. Genomic and chromosome 17 imbalances as targets for platin-chemoresistance investigation in ovarian cancer patients

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Ovarian cancer diagnosis and treatment are still one of the major

challenges for the clinical oncologist. Our hospital follows the international consensus of surgery followed by chemo, usually platinum-based in most cases. Around 30% of the patients show some kind of resistance to the chemotherapeutic approach, which is very frustrating for the patient and financially demanding for a public institution. New genomic approaches have been applied in order to find molecular markers that could predict chemotherapy response or at least to indicate possible genes or chromosome regions involved in this phenotype. The objective of this study was to evaluate chromosome regions gains and losses through comparative genomic hybridization and correlate the genetic profiling to clinical data in ovarian tumors from patients that haven't responded to cisplatin-based chemotherapy. Sixteen tumors were included, 75% with a resistant phenotype and 25% with a complete responsive phenotype. All tumors were of serous type and FIGO clinical stage III or IV. Four normal ovarian samples were taken as controls. All tumor specimens presented some degree of chromosomal imbalance widely varying from sample to sample. Resistant tumors displayed major gains in 11p12~14, 13q21~22, 16q12~qter, and repeatedly at 17q, which harbors the ERB-B2 region. Losses were found at 3q24-26, 1q31~41 and 17p. Twelve resistant samples presented some alteration at chromosome 17 which indicate that this chromosome might have "hot gene targets" for individual analysis by less expensive and less time consuming approaches that could be investigated before treatment such as mutation analysis, FISH and others.

P0537. Novel CDKN2A mutations and founder effect in melanoma families from Tuscany

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About 10% of melanoma patients have a positive family history for the disease, and a fraction of these pedigrees shows mendelian inheritance. CDKN2A is the main gene involved in genetic predisposition to melanoma, and is associated with incomplete penetrance and variable expressivity. Constitutional CDKN2A mutations are found in 15-40% of familial melanoma cases.

We have investigated the frequency and spectrum of CDKN2A mutations in a series of 18 apparently unrelated families from a region of Central Italy (Tuscany). The whole CDKN2A coding sequence was evaluated by direct sequencing. Three novel mutations were identified in 5 families. Two mutations (G23S and E27X, observed in 1 family each) are located in exon 1a, and the other one (P114S, observed in 3 families) in exon 2. Pedigrees segregating CDKN2A mutations were characterized by the presence of ≥ 3 melanoma cases, multiple melanomas, early age at diagnosis, and non-melanoma tumors (pancreatic and oral cancer). G23S is located in the functionally important first ankyrin domain of p16. A different missense mutation affecting the same codon had been reported previously and shown to disrupt p16 activity by a functional assay. Haplotype analysis in members of the 3 families segregating the G23S mutation showed a common ancestral origin of the mutation. Our data show that the G23S mutation is an important cause of hereditary melanoma in Tuscany.

P0538. FISH is a reliable method to assess the tumour content of frozen old archival material

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In order to perform genetic analysis of frozen tumour material it is important to know the actual tumour content of the available biopsy. However, the assessment of tumour content in old frozen material is often hampered by deterioration in cell morphology. To attempt to resolve this problem, prior to processing the material to molecular investigation, touch preparations were made for subsequent FISH (fluorescence in situ hybridisation) analysis. Fifty three biopsies from childhood embryonal tumours (38 nephroblastomas, 9 embryonal rhabdomyosarcomas and 6 hepatoblastomas) were available of which the oldest sample was 28 years old. A range of FISH probes were employed that represented some of the frequent cytogenetic events of these tumours. In a number of cases the karyotype was already

known and hence acted as a control for the FISH results. In 30/53 tumours an abnormal FISH result was obtained in which 21/30 (70%) indicated a tumour content of over 50%. In only two cases was no or an incomplete result obtained. For those cases where the karyotype was known no discrepancy was seen with the FISH result. In addition the signal quality from the touch preparations was superior to that typically possible with paraffin embedded material and valuable insights were gained on the karyotypic make-up of old archival material. The results demonstrate that the use of FISH is an efficient and reliable method to accurately screen the tumour content of frozen samples prior to the employment of techniques such as arrayCGH.

P0539. Clinical follow up of BRCA1 and BRCA2 carriers in the Czech Republic

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Genetic counselling and testing of BRCA1/2 genes in hereditary breast and ovarian cancer syndrome is done at the Masaryk Memorial Cancer Institute (MMCI) in Brno since 1997. Testing was done in 474 families that fulfilled our criteria, in 32.5% germline mutation in BRCA1/2 gene was discovered. Predictive testing was offered to 312 healthy relatives, in 82 women and 41 men genetic predisposition was found.

To provide carriers with a clinical follow up high-risk preventive clinic was established at the MMCI. Patients (79 women and 4 men), healthy carriers (53 women and 6 men) of BRCA1/2 mutation and high-risk individuals with undetected mutation in a family are invited regularly to check ups by a specialised nurse. Psychological support, consultation with specialised nurse, geneticist and other clinicians is available to all individuals when needed.

The role of preventive surgery is explained to all carriers. Until now 12 patients and 3 healthy carriers (age >35) underwent prophylactic adnexitomy after the genetic testing. Prophylactic mastectomy and reconstruction was done in 13 patients and 1 healthy carrier. At least 6 patients and 3 healthy carriers are decided for prophylactic surgery of breasts and 8 patients and 1 healthy carrier for adnexitomy. Psychosocial issues related to genetic testing and cancer prevention, preventive care in other centres in the Czech Republic are now investigated.

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P0540. New Croatian PTCH mutation in Gorlin syndrome family linked to craniopharyngioma

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The Nevoid Basal Cell Carcinoma Syndrome (NBCCS) or Gorlin syndrome is a rare autosomal dominant disorder characterized by multiple basal cell carcinomas (BCCs), medulloblastomas, meningiomas, fibromas of the ovaries and heart; cysts of the skin, jaws, and mesentery; pits of the palms and soles; diverse developmental abnormalities, often including rib and craniofacial alterations and less often, polydactyly, syndactyly, and spina bifida.

The syndrome is caused by mutations in the human homolog of the *Drosophila* patched gene, PTCH. PTCH is a tumor suppressor gene, located at 9q22.3, and encodes a transmembrane glycoprotein that acts as an antagonist in the Hedgehog signaling pathway. It has 12-transmembrane domains, two large extracellular loops involved in ligand binding, one large intracellular loop and intracellular N- and C-termini.

We report a family case with Gorlin syndrome associated with typical phenotypical features of widespread basocellular tumors and craniofacial and bone malformations but also with unusual appearance of craniopharyngioma. In this family we found by SSCP, dHPLC and direct sequencing a novel mutation of PTCH gene. Immunohistochemistry analyses demonstrated specific aberration of PTCH.

Our finding provide additional evidence of craniopharyngioma involvement in the pathogenesis of Gorlin syndrome.

P0541. Functional analysis of *MSH2* mutations associated with hereditary nonpolyposis colorectal cancer

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Hereditary nonpolyposis colorectal cancer (HNPCC) is associated with germline mutations in DNA mismatch repair (MMR) genes, mainly *MSH2* and *MLH1*. Most of mutations are different with each other and quite evenly distributed. Furthermore, a significant proportion of mutations give rise to single amino acid substitutions. A major challenge in the clinical management of patients with suspected HNPCC is the frequent occurrence of missense mutations, which can be considered neither deleterious nor clinically innocent à priori.

To assess their significance we studied 16 *MSH2* missense variants that occur in several subdomains of the *MSH2* protein for *in vitro* mismatch repair capability. The mutations were chosen such that the biochemical data obtained could be correlated with genetic and clinical data relating to respective HNPCC families. PCR mutagenesis was used to create the mutated *MSH2* cDNAs, the protein variants were expressed in a baculovirus expression system, and the functionality of the mutated proteins was investigated in a homologous human MMR system.

Among the sixteen *MSH2* variants, 10 variants with remarkably reduced repair efficiency were classified as defective. It is interesting to note that most of the 10 variants could be expressed only in lower amounts than the wild type protein suggesting that these amino acid substitutions impart also instability on the mutant proteins. Six variants acted like wild type protein in the repair assay and further examinations need to be done to evaluate their pathogenicity.

P0542. Analysis of chromosomal translocations involved *MLL* gene using hybridization with oligonucleotide microarrays.

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11q23 chromosomal rearrangements occur in 70-80% of ALL cases and in 50-60% of AML cases among infants and traditionally are characterized by poor prognosis. However, last investigations demonstrate the noticeable clinical heterogeneity of patients regarding to a gene-partner involved in rearrangements with *MLL*- gene. The diversity of genes (*MLL*-gene has more than 30 different gene-partners) makes difficult the analysis of 11q23 abnormalities using standard methods (cytogenetics, FISH or standard RT-PCR).

Here we present a biochip, which allows to identify 7 chromosomal aberrations with *MLL* gene: t(4;11), t(6;11), t(9;11), t(10;11), t(11;19)*ENL*, t(11;19)*ELL*, dup(11). The *MLL*-biochip consists of 16 types of oligonucleotide probes, representing parts of gene sequences involved in aberrations, and allows the analysis of about 45 fusion transcript variants.

The 82 bone marrow or peripheral blood samples from patients with primary AML and the 83 samples from patients with primary ALL were analyzed using RT-PCR with hybridization on biochips. We have found that translocations involved *MLL*-gene, occur in 67% of infants with AML, in 8 % of children older than 1 year with AML, in 48% of infants with ALL and in 9% of children with ALL aged 1 to 3 years. In the present investigation the dup(11) was not included in the analysis since during screening using standard RT-PCR approach we detected the *MLL* duplication more often than it was described previously for leukemia patients. We supposed that the dup(11) might be detected in low titer in the majority of patients but this fact had no clinical significance.

P0543. *TNFα* promoter SNPs in Croatian population - association with colon cancer

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The immune system is a complex network of cells that are involved in the protection of humans against infectious agents and tumor growth. Inherited polymorphisms in immuno-modulatory genes may contribute to variation in immune function and genetic susceptibility for complex

disease, including cancer.

TNFα is a cytokine produced by activated monocytes and macrophages, which play a key role in the inflammatory response. Increased serum *TNFα* levels have been described in cancer patients, and are associated with an adverse disease outcome. *TNFα* gene is mapped to chromosome 6p21.3 and a large number of polymorphisms of its promoter, called "high-production" polymorphisms, have been described. The aim of our study was to estimate allelic frequency for four promoter SNPs in *TNFα* gene, -238, -308, -857 and -1031 in the Croatian population and in patients with colon cancer. DNAs obtained from 150 colon cancer patients and 150 unrelated healthy volunteers were genotyped for the *TNFα* -238, -308, -857 and -1031 SNPs using real-time PCR TaqMan® SNP genotyping assays. The frequency of alleles associated with altered *TNFα* production was: 3%, 4%, 18.8% and 18.8% for -238A, -308A, -857T and -1031C, respectively. "High-production" allele variant -308A was more common in the population with colon cancer in the comparison to healthy volunteers. The association between these polymorphism and colon cancer has to be investigated in the future studies.

P0544. Differential activity of demethylating agents on the *MLH1* promoter.

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Regulatory genes are often hypermethylated at their promoter regions and silenced in cancer. Therefore, the use of demethylating agents, that could reactivate these genes, appears as a logical way to approach cancer treatment. The most widely used demethylating agent is 5-aza-2'-deoxycytidine (5-aza-dC), a nucleoside analogue, that, unfortunately, exhibits large side effects. This has prompted to find out less toxic agents. Procainamide, a non-nucleoside inhibitor of DNA methyltransferases used in the clinical setting for the treatment of cardiac arrhythmias, induced demethylation and re-expression of several genes in different cell lines.

Our purpose was to evaluate whether procainamide can be used to reactivate *MLH1* in human colorectal cancer cell lines (HCA-7 and RKO) with *MLH1* promoter hypermethylation. The compound was administered for 24h - 14 days at 50-1000 µM concentration. *MLH1* gene expression was evaluated by Real-Time PCR. No RNA could be detected at any drug concentration and time points tested in our cells. As a positive control 5-aza-dC was also used and, as expected, it restored *MLH1* expression. To establish that our procainamide treatment regimen was effective on other promoters, we showed an up-regulation of *RARβ* expression in the MCF-7 cell line following treatment at 500 µmol/L for 3 days.

In conclusion, our results show that the effects of demethylating agents can vary among different cell lines and genes, suggesting potential limitations to the efficacy of these compounds.

P0545. A novel RFLP analysis of mitochondrial D310 polymorphism in breast and colorectal cancers

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Recently a homopolymeric C stretch (D310) was identified in the D-loop region of mitochondrial DNA as a hot spot of mutations. This region consists of two cytosine stretches interrupted by a thymine nucleotide. It is widely accepted that 7 cytosine residues are located at the first stretch but, it is highly polymorphic between 6-C to 9-C. We conducted an RFLP analysis using restriction enzyme, BsaXI, in order to determine the distribution of 7-C sequence among 41 healthy persons and 50 cancer patients in the Turkish population. We found out that 65.9% of healthy individuals showed BsaXI (-) genotype that meant most of the population did not fit into the 7-C sequence. No heteroplasmy was detected among the healthy individuals. Heteroplasmy was encountered in breast and colorectal patients (8 and 24%, respectively). No significant difference was found between the matched samples of tumor and normal tissues in their BsaXI status. BsaXI status of colorectal cancer samples were significantly different from that in healthy individuals. In conclusion, BsaXI RFLP analysis is

a rapid and a non-radioactive method for the determination of D-310 polymorphism and may be a useful tool for early tumor detection.

P0546. Significance of additional chromosomal aberrations in bone marrow cells of children with TEL/AML1 positive acute lymphocytic leukemia

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Cryptic translocation t(12;21)(p13;q22) which give origin to the hybrid gene TEL/AML1 can be found by FISH in approximately 25% of children with acute lymphocytic leukemia (ALL) as the most frequent specific aberration. Despite of the fact that according to the most authors this finding is favorable prognostic sign, TEL/AML1 positive children can have late relapses. One of the reasons could be high instability of the genome of leukemic cells, which is manifested on chromosomal level by additional aberrations and complex rearrangements. As it was already proved in adult patients with acute myeloid leukemia or other hematologic malignancies, complex karyotypes in bone marrow cells mean adverse prognostic effect. The same could apply for pediatric patients.

We examined 79 children with ALL and TEL/AML1 fusion gene proved by RT-PCR. Most of them are living in the first or second complete remission. Relapse appeared in 16 children (20%). Two patients died (one because of relapse and second for treatment complications). Karyotypes were analyzed by conventional and molecular-cytogenetic methods (I-FISH, WCP-FISH, mFISH). In 48 children (61%) we found except t(12;21)(p13;q22) additional chromosomal aberrations, the most frequently trisomy or tetrasomy of chromosome 21 (14 cases), deletion of non-translocated TEL allele (14 cases) and/or deletion of 6q (6 cases). In seven children variant translocations of chromosomes 12 and 21 with other partners were found and in 35 children (44,3%) complex aberrations were identified. In our cohort additional aberrations were indicator of poor prognosis ($p=0,05$).

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P0547. Molecular cytogenetic analyses of malignant brain tumour cells.

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Diffuse gliomas are heterogeneous group of central nervous system tumours with various histological subtypes that differ in their response to treatment and prognosis. Differentiation of glial subtypes based solely on morphology is subjective. Therefore, new diagnostic and prognostic indicators must be sought to enable stratification of the treatment and to help reduce morbidity and mortality of patients. One possibility is subclassification of patients according to findings of specific chromosomal aberrations.

For detection of most frequent chromosomal changes in glial cells (deletion of tumor-suppressor genes p53, p16 and RB1, deletion of 1p36 and/or 19q13.3, amplification of EGFR gene, trisomy 7 and monosomy 10) we used I-FISH with locus-specific and/or α -satellite probes (Abbott Vysis™). We examined 20 patients with different types of gliomas (4x low-grade astrocytoma, 4x anaplastic astrocytoma, 6x glioblastoma, 2x low-grade oligodendrogloma, 4x anaplastic oligodendrogloma). The results of molecular-cytogenetic analyses were correlated with morphological and clinical findings. In two patients with original diagnosis of anaplastic astrocytoma (grade III) we proved amplification of EGFR gene - typical aberration of glioblastoma (grade

IV). In other case with oligodendrogloma (grade II) we proved deletion of RB1 gene - typical finding in high grade astrocytoma. In four patients with anaplastic astrocytoma combined deletion of 1p36 and 19q13.3 was found, which could predict good response to chemotherapy in these patients.

I-FISH is a powerful tool for surveying chromosomal aberrations in tumour cells. A systematic molecular cytogenetic analysis may advance diagnosis, grading and classification of brain tumours.

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P0548. Investigation of the genetic basis of the outcome of chemotherapy in breast cancer patients.

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Pharmacogenetics aims to identify the inherited basis for inter-individual differences in drug response. Polymorphisms contributing to this inter-individual variation may be located in genes encoding drug metabolizing enzymes (DME), drug transporters (DT), receptors, DNA repair enzymes, cell cycle regulators (CCR), and other potential drug targets. To draw conclusion on the relationship between the genome and chemotherapy outcome of breast cancer patients genotype and survival data are needed. This requires patients with long follow-up and their formalin-fixed paraffin-embedded tissues (FFPET). Constitutional DNA was isolated from FFPE of 199 breast cancer patients who received adjuvant chemotherapy (CMF, and anthracyclines), and had at least 5 years documented clinical follow-up. There were 100 patients without and 99 patients with relapse. Genotypes were established by MALDI-TOF MS at 32 polymorphic loci of potentially relevant genes. We applied univariate analysis for genotype associated over all survival (OVS). All genotype frequencies were in Hardy-Weinberg equilibrium. We observed significant differences in genotype distributions with respect to OVS at three loci: CCNA2_159_A>G ($p=0.0138$), GSTP1_313_A>G ($p=0.0192$), XRCC3_11672_C>T ($p=0.0062$). These data provide a first basis for the identification of constitutional markers potentially useful for the prediction of chemotherapy outcome in breast cancer.

P0549. Results of BRCA1 and BRCA2 mutation analysis in the Czech breast/ovarian cancer patients

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Germline mutations in highly penetrant cancer susceptibility genes BRCA1 and BRCA2 cause genetic predisposition to breast and ovarian cancers.

Molecular genetic analysis of BRCA1 and BRCA2 genes in 359 high-risk breast and breast/ovarian cancer families, 32 patients with bilateral breast or ovarian cancer or both, and 83 women diagnosed with early-onset sporadic breast/ovarian cancer below 40 years has been performed in our laboratory since 1999. Overall, in 32.5% of tested index cases pathogenic mutation was discovered.

Concerning disease causing mutations, 45 different germline mutations were found, 22 in BRCA1 gene and 23 in BRCA2 gene. The most frequent mutations were c.5385dupC (42 cases), c.3819_3823delGTAAA (14 cases), c.300T>G (13 cases) in BRCA1 gene; and c.8138_8142delCCTTT (10 cases), c.8765_8766delAG (10 cases) in BRCA2 gene (using the BIC Database numbering). Altogether, these 5 mutations represented 58% of all detected mutations.

In addition to the pathogenic mutations, 26 different variants of unknown significance (12 in BRCA1 gene and 14 in BRCA2 gene) were found. The population frequency of these variants and segregation analysis was studied.

Moreover, our predictive testing of 342 symptomatic and nonsymptomatic relatives revealed 145 mutation carriers. Preventive care in a specialised clinic is offered to all of them.

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P0550. Quantitative Image Analysis for Scoring Her2 Amplification Status in Metastatic Breast Carcinoma.

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We examined paraffin wax sections from 114 cases of metastatic breast cancer in order to compare the amplification status of the oncogene Her2 by means of an immunohistochemical (IHC) stain test (HercepTest) against FISH using the Abbott-Vysis PathVysion probe kit.

The Her2 Digital Scoring Application of Applied Imaging's Micrometastasis Detection System (MDS) was used in addition to manual scoring of FISH and HercepTest to determine if this system provides an accurate alternative.

Of the IHC 3+ cases, 90% showed Her2 amplification by FISH. Of the IHC 2+ cases, 32% showed amplification by FISH. Of the negative IHC cases (0 or 1+), 6 % were amplified by FISH. Classification discrepancies were observed in 18% of HercepTest cases scored by eye and by using the MDS system. The MDS proved to be consistent with manual FISH scoring and correctly differentiated most cases of ambiguous manual IHC scoring.

Published response data has shown that the determination of Her2 amplification by FISH is a more reliable selection procedure for Herceptin treatment than HercepTest. We have found that the MDS system does provide an accurate alternative to manual FISH scoring and that it also appears to improve the correlation between IHC and FISH categorisation.

In addition, we have also presented an interesting case with heterogeneous tumour populations where FISH analysis has called into question the DakoCytomation HercepTest scoring criteria.

P0551. Detection of chromosomal changes in plasma cells by interphase fluorescence in situ hybridization in patients with multiple myeloma

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Multiple myeloma (MM) is hematological malignancy characterized by the infiltration of bone marrow by plasma cells with numerical and structural chromosomal abnormalities. The most frequent are deletions of RB1 gene at 13q14 or monosomy 13 and translocations involving 14q32, where is the locus for immunoglobulin heavy chain (IgH). From different partner chromosomes in translocations, 11q13 is the most frequent one, giving better prognosis. FISH analysis of plasma cells labeled by immunofluorescence allows their identification even in cases with low bone marrow infiltration.

The aim of the study is identification of the frequency of deletions and/or monosomies 13, 14q32 rearrangements, t(11;14) and evaluation of their prognostic significance.

We examined bone marrow samples of patients with MM by FISH with specific DNA probes (Abbott-Vysis™). Identification of deletion/monosomy of chromosome 13, was done by locus specific probes LSI 13 (RB1) 13q14 and LSI 13q34, 14q32 translocations were verified with LSI IgH rearrangement probe, t(11;14)(q13;q32) was detected with LSI IgH/CCND1 probe. Totally 66 patients were examined with 13q14/13q34 probes, deletion of RB-1 gene was found in 22 (33%) and monosomy 13 was identified in 20 (30%), combination of both was proved in five (7,6%). IgH translocation was evaluated in 58 patients and was detected in 21 (36%) of them (deletions, partial trisomies and monosomies were also found). Five out of 10 patients examined for t(11;14) were positive.

Prognostic significance of chromosomal changes and the contribution of immunofluorescence in cytogenetic examination of MM will be evaluated.

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P0552. Second hit analysis in tumors from HNPCC patients carrying novel large genomic deletions in MSH2 or MLH1

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Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominantly inherited cancer predisposition syndrome caused by germline mutations in DNA mismatch repair (MMR) genes. Large genomic deletions in MSH2 or MLH1 have been reported to account for up to 27% of all MMR gene mutations, missed by conventional mutation detection techniques.

We screened for large genomic deletions/insertions in the germline using the multiplex ligation dependent probe amplification (MLPA) assay in 21 unrelated HNPCC patients whose tumors exhibited microsatellite instability and showed immunohistological loss of either MLH1 (n=12) or MSH2 (n=9) but in whom no pathogenic MMR gene mutation could be identified. We detected four novel large genomic deletions, two in MLH1 (EX7_9del and EX13del) and two in MSH2 (EX7_8del and EX8_11del).

To assess the type of the second, somatic alteration (second hit) occurring in the tumors, we analysed 8 different cancers (5 colorectal, 2 renal and 1 brain tumor) from these deletion carriers using microsatellite markers and the MLPA assay. Assessing allelic imbalance by marker analysis was impeded by the presence of microsatellite instability in about 50% of informative markers whereas the MLPA assay allowed to determine exon-specific gene dosage in 7 out of 8 cancers. Consequently, none of the tumors showed evidence for whole gene loss. In two colorectal cancers (both from the MLH1 EX7_9del kindred) as well as in the brain tumor (MSH2 EX8_11del) the second hit was identical to the first hit alteration, indicating mitotic recombination as primary mechanism of somatic inactivation in these tumors.

P0553. Increasing detection efficiency of microsatellite instabilities in colon carcinoma by applying a label-free method

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Microsatellite instability (MSI) is caused by a failure of the DNA mismatch repair system and occurs frequently in various types of cancer. Since MSI, associated with ca. 10 to 15 % of colorectal, gastric or endometrial carcinoma, impact clinical prognosis, MSI analysis is an important tool of molecular pathology. This study aimed to develop a simple and efficient procedure of MSI detection. 40 cases with no (27), low (1) or high (13), pre-identified by conventional fluorochrome-associated PAGE technology, were selected out of a panel of 150 patients with colon carcinoma. Microdissected non-tumor (N) and tumor (T) tissue areas of one or two 4 µm-sections were deparaffinized and DNA was extracted. Primer sequences recognizing the five microsatellite loci BAT25, BAT26, D5S346, D17S250, D2S123, were selected according to the recommendation of the 1997 National Cancer Institute-sponsored conference on MSI. Primer sets were applied in label-free duplex or single PCR assays for DNA amplification and amplicons were analysed by microfluidics based on-chip electrophoresis. In all 40 cases, chip linked microcapillary electrophoresis of the amplicons, arisen from tumor and non-tumor DNA, resulted in highly resolved, distinct patterns of each of the microsatellite loci. Label-free detection of MSI could be demonstrated by microsatellite loci-associated deviations in the electropherogram profiles of tumor and non-tumor material, and confirmed the prediagnosis of the MSI cases by conventional technology. Here, we present a simple and robust approach for MSI detection, which allows a label-free microsatellite analysis of uncharacterized microdissected tissue areas within 30 minutes.

P0554. RNASEL R462Q is significantly associated with Prostate Cancer in the Italian population

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RNASEL is the most likely candidate prostate cancer susceptibility gene of the HPC1 locus at 1q23. It encodes a constitutively expressed latent 2'-5'-oligoadenylate-dependent ribonuclease that mediates the antiviral and pro-apoptotic activities of the interferon-inducible 2'-5A system.

In single high risk families rare inactivating mutations have been reported and association studies of sporadic prostate cancer with the common R462Q polymorphism resulted in variable findings in different populations. To our knowledge, this putative low-penetrance allele has not been previously analyzed in the Italian population.

We collected DNA samples of 192 prostate cancer patients attending four participating Urologic Units and 218 controls, with the aim to evaluate the frequency of R462Q in our population and detect possible differences between sporadic and familial cases, useful to plan a larger prospective study on this and other SNPs. Genotyping was done by PCR-DHPLC.

Genotype frequencies (%) in controls were: RR 43.6, RQ 42.2, QQ 14.2 vs 31.2, 51.6 and 17.2 in patients. Allele Q was significantly more frequent in patients (69%) vs. controls (56% p<0.01), and RR have an average age of onset three years later than patients carrying Q in single or double dose.

Early detection of prostate cancer is currently based on PSA testing, which as a screening strategy is debated. Identification of new predisposing genes might contribute both to a better understanding of the underlying molecular mechanisms, and also open new ways for defining the individual risks, a difficult challenge if prostate cancer susceptibility depends on several low-risk rather than few high-risk genes.

P0555. A Report of Cytogenetic Study in 146 Serbian Children with ALL and its Prognostic Value

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In this study we present the results of cytogenetic analysis in 146 children with ALL, diagnosed between 1998 and 2003, at the Mother and Child Care Institute of Serbia "Dr Vukan Cupic". Patients, all under 15 years old, belonged to various ALL types (L1 or L2). There were more males patients than females (1,78 : 1).

Normal karyotype was found in 79 (54%) of our patients. Chromosomal abnormalities were seen in 67 (46%) children; among them 44 (65%) have numerical and 23 (35%) structural rearrangements. Structural abnormalities were presented in a form of specific and nonspecific chromosomal aberrations. Among specific chromosomal disorders cytogenetic analysis revealed: hyperdiploidy in 37 (55.7%) children with ALL L1 or L2, hyperhaploidy in one patient with ALL (L1), translocation 9/22 [t(9;22)(q34;q11)] in 4 (6%) patients with ALL (L1 or L2), translocation 4/11 [t(4;11)(q21;q23)] in one patient with ALL (L1) and translocation 11/14 [t(11;14)(p13;q11)] in one patient with the same type of ALL. Nonspecific chromosomal abnormalities detected in our group of patients were: hypodiploidy (6 patients (9%) with ALL(L1 or L2)); translocations: t(6;9)(q23;p24) (1 patient with ALL (L1)), t(13;19)(q14;p13) (1 case with ALL(L1)); deletions: del(7)(q22) (1 patient with ALL(L1)), del(8)(p11) (1 case with ALL (L2)), del(2)(q33) (1 patient with ALL (L2)) and inversion of chromosome 1 [inv(1)(p34;q21)] in one patient with ALL (L1).

The authors will discuss the prognostic value of nonspecific rearrangements in analysed sample of ALL patients.

P0556. Analysis of genetic events in the 17p13 region points to monoclonal origin of multifocal and recurrent bladder cancer.

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Bladder cancer is the second most common malignancy of the genitourinary tract with about 90% of tumours belonging to transitional cell carcinoma (TCC). Approximately 70% of patients with superficial TCC develop recurrences after transurethral resection, and nearly 30% of patients present at diagnosis with multifocal disease. Synchronous or metachronous development of superficial TCCs evokes the question of clonal nature of these tumours. Published studies present evidence for both the monoclonality hypothesis (which proposes that malignant cells spread via intraluminal seeding or intraepithelial migration), and the field cancerisation hypothesis (which proposes transformation of numerous urothelial cells at multiple sites due to exposure and

accumulation of carcinogenic events).

We attempted to address this question by the analysis of genetic events in the 17p13 region in multifocal and recurrent superficial TCCs. TP53 gene mutations and loss of heterozygosity (LOH) of three intragenic and two extragenic TP53 polymorphic DNA markers were tested in 25 patients with 2-8 TCCs sampled between 2000 and 2004 (a total of 83 tumours). We found that the tumours from an individual always showed the same TP53 mutation (4 of 25 patients (16%)). In similar, we found that with the exception of two patients with discordant findings and three non-informative patients, the tumours from each individual always showed either absence or presence of LOH, with the same allele lost in all tumours (14 of 20 patients (70%), and 6 of 20 patients (30%), respectively). These results rather support the monoclonality hypothesis for the development of multifocal and recurrent superficial TCCs.

P0557. Expression profiling of T-cell lymphomas defines genetic determinants of survival and response to treatment related genes.

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T-cell non-Hodgkin's lymphomas (T-NHLs) constitute an heterogeneous group. They are in general aggressive tumours with clinical behaviour that may not correlate with morphology. In a previous study (1) we tried to subclassify this group by use of cDNA microarrays and found that according to the mean survival (10 months) we could establish a common genetic signature with 5 genes related to survival and drug response. The aim of the present study was to validate these results in a new series and to identify genes related to survival and response to treatment in T-NHLs. Expression profiling of T-NHLs were established using a cDNA microarray, containing 6386 cancer related genes, developed by the CNIO. RNA from 60 T-NHLs was obtained and subsequently cDNA and T7-amplified RNA was synthesised. T-lymphocytes extracted from peripheral blood, thymus samples or reactive node tissues were used as control tissues.

Previous results were validated and furthermore, cluster analysis identified genes that were significantly over or under-expressed, most of them of immune response, genes involved in the NFkB pathway, cell cycle or T cell differentiation. Importantly, we found two different subgroups of PTCLs based on the expression of NFkB pathway genes. A reduced expression of NFkB pathway genes was significantly associated with shorter survival, in contrast to longer survival times showed by the NFkB+ lymphomas. Over-expression of two representative genes of the NFkB pathway, NIK and IKBa, was found among NFkB+ lymphomas. These genes might be considered as targets for new therapies.

1- Martinez et al. Clin Cancer Res 2004

P0558. Study of mtDNA amount in prostate cancer by real-tyme PCR.

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According to recent estimates of the global cancer prevalence, prostate cancer (PC) is the third most common cancer in men, causing 40,000 death per year both in Europe and the United States.

In recent years, a high number of point mutations, insertions or deletions in mtDNA have been identified in multiple cancers. The analysis of the mtDNA has been suggested as a very useful tool for the early detection of cancer.

We studied the relative amount of mtDNA in normal, tumoral and hyperplastic samples corresponding to 30 patients with PC and 5 controls. Small cylinders of prostatic tissue were obtained from radical prostatectomy specimens. In addition, 7 seminal vesicles from patients and controls were also studied as a second control tissue because it seldom, very infrequently malignizes.

Our purpose was to determine if there were differences in the mtDNA quantity between the different types of samples or between patients and controls. We used a real-time PCR technique for the amplification of the mitochondrial gene *ND2* and the nuclear housekeeping gene *18S*.

There were no statistical significant differences between normal, tumoral, hyperplastic or seminal vesicle groups, nor between patients and controls.

P0559. Germ-line mutations and large deletions of the APC gene in Czech FAP patients

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Germ-line APC (adenomatous polyposis coli gene) mutations are responsible for familial adenomatous polyposis (FAP). FAP is a dominantly inherited predisposition to colorectal tumors, which shows substantial phenotypic variability. Routine mutation detection techniques fail to detect APC germ-line mutations in 20 - 40% of patients with classical or attenuated FAP. The failure to detect germ-line mutations might be caused by large deletions, MYH mutations or by mutations in other genes. From a series of 103 Czech unrelated probands with colorectal polyposis, 72 (69,9%) were found to have truncating or missense mutations. Using PCR, PTT, DGGE methods and sequence analysis, the 39 previously reported and 33 mutations unique for Czech populations were detected. The APC mutation negative patients have been screened for large APC deletions using multiplex ligation dependent probe amplification (MLPA). Two whole-gene deletions and one exon 14 deletion were found in a set of the 37 APC negative probands. Fine mapping has to be performed to assess the exact molecular extent of the APC germ-line deletions identified. Supported by grant project IGA MZ CR NR8103-3/2004.

P0560. Novel 3' mutation of the APC gene in a patient with isolated desmoid tumors in a family suffering from familial adenomatous polyposis (FAP).

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Desmoid tumors occur sporadically or as part of the extraintestinal manifestations of FAP, the phenotypic expression of APC gene mutations. Considerable clinical variability is known both within and especially between families. Some phenotype-genotype correlations concerning the gastrointestinal and extra-gastrointestinal manifestations of the disease have been demonstrated. In particular, 3' mutations of the APC gene may result in attenuated adenomatous forms or, in extreme cases the occurrence of desmoid tumors only. We present here the case of a 42-year-old woman suffering from desmoids tumors without colonic involvement but for whom the history of FAP among siblings indicate APC gene mutation screening. A frameshift mutation due to a single adenine deletion at position 5772 (codon 1924), located at the 3' end of exon 15 of the APC gene was found. Six other families in whom desmoid tumors represent the main phenotype and mode of ascertainment have been described in the literature. All the mutations were found in the 3' end of the APC gene. Desmoid tumors were found in 79 % of patients, FAP in only 37 % and mainly in its attenuated form, and colorectal cancer may be the only colonic manifestation in some individuals. This data suggests that an APC gene mutation must be looked for in cases of familial desmoid tumors, even in the absence of a personal or familial history of FAP. Likewise, colonoscopy must be performed in the case of a desmoid tumor, even when sporadic, in order to detect FAP and its risk of colorectal cancer.

P0561. Induction of apoptosis in BCLL cells by fatty acids

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Histone deacetylases (HDACs) are a group of enzymes able to change nucleosome structure by removing acetyl groups from histone tails. This modification results in more compact chromatin structure. Inhibition of HDACs in neoplastic cells leads to change in some genes expression (2-5% of cellular genes) and, subsequently, differentiation or apoptosis. Thus HDAC inhibitors possess antitumour activity and, since they are well tolerated, create a new strategy in cancer treatment.

Short chain fatty acids and some of their derivatives form a class of substances who work as histone deacetylase inhibitors.

In our study we cultured B-CLL (B-cell Chronic Lymphatic Leukaemia)

cells obtained from 20 patients in liquid media with sodium butyrate and butyrate derivative, phenylbutyrate. After 24 hours of culture we observed significant increase in number of apoptotic cells using anti active caspase 3 antibody in flow cytometer, in comparison to control cultures (without fatty acids). The intensity of apoptosis was compared to positive control (with dexamethasone).

In conclusion, the apoptotic properties of fatty acids alongside their low toxicity suggest that they could be used in modern schemes of B-CLL treatment.

P0562. Methylation profile of N33, HIC, GSTP1 and CDKN2 at different stages of prostate carcinogenesis.

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DNA methylation of CpG sites of tumor suppressor genes is a frequent epigenetic event in the cancer pathogenesis. We examined the methylation profile of N33, HIC, GSTP1 and CDKN2 genes in the spectrum of prostate cancer disease progression, from benign prostate hyperplasia (BPH) and prostatic intraepithelial neoplasia (PIN) to primary prostate cancer. Biopsy specimens were obtained from patients with BPH (n=24), PIN (n=32) and adenocarcinoma (n=52), 28 prostate tissues were obtained from patients with prostate cancer after radical prostatectomy. Methylation status was determined using methylation-sensitive PCR.

Methylation frequency of HIC1 in BPH samples was 37% and methylation frequencies of PIN and adenocarcinoma were significantly higher 66% and 65% accordingly (P=0,05). Promoter hypermethylation of p16 was detected in 25% of hyperplasia, 29% of neoplasia and 36% of carcinoma. The similar pattern of methylation was shown for GSTP1 gene (25%, 16% and 42%). It was interesting that methylation of N33 was absent in the preinvasive lesions but was frequent in adenocarcinoma (18%, P=0,02). Methylation percentages in prostatectomy tissues were: GSTP1, 32%; HIC1, 71%; p16, 18%; N33, 20%. There were no significant differences in the frequencies of methylation in biopsy and prostatectomy prostate adenocarcinomas specimens for individual genes.

We can conclude that N33 methylation is related to late, tumor-associated events, while HIC1, GSTP1 and CDKN2 methylation appears with different frequencies at cancer precursor lesions. These early epigenetic DNA alterations are the accessory component of extensive tumour reorganizations and provide clinically useful markers to diagnose early prostate cancer lesions and assess disease prognosis.

P0563. The survival of human B-CLL cells and "nurse-like" cells in the different conditions of culture in vitro.

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Chronic lymphocytic leukemia (CLL) is a disease characterized by an excessive accumulation of B lymphocytes displaying deficient programmed cell death and a different clinical course and prognosis.

The aim of investigation: To examine the capacity of leukemic lymphocytes and "nurse-like" cells to survive *in vitro* and protective effects of "nurse-like" cells on leukemic lymphocytes.

Object and methods: The lymphocytes isolated from peripheral blood obtained from patient with B-CLL were examined. The cells were cultured under four separate conditions: 1) medium alone, 2) medium supplemented by apoptosis-inducing dexamethasone, 3) medium conditioned by coculturing leukemic lymphocytes and "nurse-like" cells, 4) medium conditioned by coculturing leukemic lymphocytes and "nurse-like" cells and supplemented by apoptosis-inducing dexamethasone. The cells were quantified by flow cytometry after labelling with FDA and propidium iodide / annexine-V.

Results: After 14 days of culture *in vitro* we have demonstrated increased survival of lymphocytes in the presence of "nurse-like" cells (96.35 vs 93.15% for cultures without dexamethasone and 67.80 vs 50.91% for cultures with this factor). We also have noticed decreased capacity of "nurse-like" cells to survive as compared with leukemic lymphocytes (83.02 vs 96.35% for cultures without dexamethasone and 1.60 vs 67.80% for cultures with this factor).

Conclusions: This experiment could show the protective role of "nurse-like" cells in the apoptosis of CLL cells under different conditions of culture *in vitro* and decreased resistance to dexamethasone-induced apoptosis of these cells.

Because of the fact that it was an initial study further ones on the interactions between lymphocytes and "nurse-like" cells are needed.

P0564. Familial Nonmedullary Thyroid Carcinoma In Italian Families: Clinical And Genetic Heterogeneity

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The familiar form of nonmedullary thyroid carcinoma (FNMTC) is a complex genetic disorder characterized by bilateral and multifocal thyroid cancer, occurring at an earlier age and with a more aggressive course than the sporadic forms, often associated with benign thyroid pathologies. Several environmental risk factors have been identified, but little is known about genetic risk factors of the disease. The finding of RET proto-oncogene mutations cosegregating with papillary thyroid cancer suggests an important role of this gene in epithelial thyroid carcinogenesis. In addition, a recent linkage study identified a novel FNMTC susceptibility locus on chromosome 2q21. We describe here five large Italian families with familial aggregation of PTC and benign thyroid disease. Twelve individuals had papillary thyroid cancer while 27 family members showed benign thyroid disease, either plurinodular goiter or thyroiditis. Mutational analysis of the RET proto-oncogene failed to identify pathogenic mutations in all families. Moreover, in the two families with at least three individuals with PTC, linkage to the FNMTC locus was excluded. Our findings indicate that different genes are involved in the pathogenesis of the familiar form of nonmedullary thyroid carcinoma, further confirming genetic heterogeneity of this condition.

P0565. High frequency of p16^{INK4b} (MTS1) gene promoter methylation in Iranian patients with breast tumor

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The methylation status of p16^{INK4b} (MTS1) gene promoter was analyzed in 67 breast tumors from Iranian patients with breast cancer. The analysis was performed using methylation-specific polymerase chain reaction (PCR) (MSP) followed by restriction-enzyme related PCR (REP). Methylation of the p16 gene was detected in 47% of the breast tumors analyzed. The data showed that the tumors with high-grade (85%) were more often methylated than those with low-grade (27%). Moreover, a large variation in the methylation patterns of p16 exon 1 was observed from one sample to another. Analysis of the expression of p16 promoter in methylated and non-methylated tumors indicated the association of methylation and the absence of p16 gene expression. The results suggested a role for p16 gene promoter methylation during breast tumorigenesis and a possible inactivation of p16 expression in high-grade breast cancers.

P0566. Familial breast cancer- two mutations, two phenotypes.

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The co-existence in an individual of BRCA1 and BRCA2 mutations is unusual. Initially it was believed that the homozygous or compound heterozygous state of BRCA1 and/or BRCA2 was lethal. Reports now exist of such compound heterozygotes.

We have encountered a family where two sisters have co-existent BRCA1 and BRCA2 mutations. The proband aged 58 had bilateral breast cancer (aged 34 and 53) and colorectal cancer (aged 39 and 58 years). Her sister aged 63 has had regular cancer surveillance without the detection of malignant pathology thus far. The pattern of cancer in this kindred is consistent with a predisposing mutation in a BRCA gene. (BRCA PRO scores: A1 - 0.885; A2 - 0.110 Combined score -

0.990) In a routine familial cancer clinic, 2 mutations were identified in the proband (BRCA 1 - frameshift mutation in exon 11 nt3450delCAAG STOP 115; BRCA 2 - splice site mutation IVS7+2 T>G in exon 7) Immunohistochemistry of the bowel cancer showed normal expression for the mismatch repair genes.

Predictive testing was offered to her asymptomatic sister, who was found to be positive for both mutations.

While it is acknowledged that the penetrance of each mutation within BRCA1 or 2 is incomplete, the very different phenotypes of these sisters if of interest.

Studies of their demographic differences have given few insights into the differing clinical outcomes. Modifier genes or haplotypes are a plausible explanation. Larger family studies are underway to eliminate other possible genotypic differences which account for this discordance.

P0567. Genetics of Diagnosis and Management in Chronic Myeloid Leukemia (CML)- An Indian study

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Chronic Myeloid Leukemia (CML) is a hematopoietic malignancy characterized by presence of Philadelphia (Ph¹) chromosome and/or *bcr/abl* fusion gene. Although CML has been widely studied, various genetic changes leading to progression of disease and relapse are still unexplored. The present study was conducted to evaluate cytogenetic and molecular anomalies and response to therapy in CML patients on various therapies (IFN- α 2b+HU, IFN- α 2b+HU+Ara-C, ST1571 and allogeneic BMT).

Six hundred bone marrow samples from 349 suspected CML patients were analyzed using conventional cytogenetics and Fluorescence In Situ Hybridization (FISH) analysis. Of these, 101 cases on the above therapies were followed at sequential intervals of 4-6 months for four years. Probes used for FISH analysis were specific for *bcr* and *abl* genes and centromeres of chromosomes X and Y (in cases of sex-mismatched BMT). Novel cytogenetic and molecular anomalies could be identified at diagnosis and during therapies that were found to be associated with specific clinical/hematological profiles. Appearance of some anomalies was followed by clinical/hematological relapse and progression to blast crisis. New variant Ph¹ translocations associated with resistance to therapy were also observed. Minimal residual disease (MRD) could be evaluated using FISH analysis in cases with complete cytogenetic response.

These findings reiterate that molecular analysis in conjunction with cytogenetic analysis has tremendous importance not only in accurate diagnosis and management of CML but also in predicting relapse/progression. Further identification of novel anomalies indicate the involvement of chromosomes other than 9 and 22 in the pathogenesis of CML, which needs to be explored further.

P0568. No sib pair concordance for breast or ovarian cancer in BRCA1 mutation carriers

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Modifying factor(s) of expression of BRCA1 mutations would be expected to induce concordance for breast and ovarian cancer in affected sibships. We identified all pairs with cancers where one or both sisters were demonstrated to carry a BRCA1 mutation.

The preliminary results were that under the assumption of random distribution we would expect 23.4 pairs to be concordant for breast cancer, and the observed number of concordant pairs were 22 ($p < 0.05$). The expected number of concordant pairs for ovarian cancer were 10.4 and the observed number 9 ($p < 0.05$). Final results of the study will be given. Methodological problems will be discussed. In conclusion, there may be no single major modifying factor of expression of BRCA1 mutations. The mutations did not differ with respect to breast or ovarian cancer as clinical expression. Previous disease manifestations in close relatives may have no bearing on first cancer to be expected in a young female mutation carrier. This means that, in BRCA1 carrying kindreds without ovarian cancer, the risk for ovarian cancer may be as high as in ovarian cancer kindreds. It means that, in ovarian cancer kindred, the next young cancer to expect may

be breast cancer because it occurs at an earlier age. It also means that research strategies to uncover modifiers of BRCA1 expression, may be suitable to uncover multifactorial systems and not only single factors.

P0569. A preliminary molecular analysis of APC gene in the Iranian FAP patients

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Germ-line mutations in the adenomatous polyposis coli (APC) gene are causative for familial adenomatous polyposis (FAP), a dominant inherited syndrome characterized by presence of multiple adenomatous polyps in the colon and rectum. In this study, we screened the coding region of APC gene for germ-line mutations in seven Iranian FAP patients. Clinically typical FAP was diagnosed in three patients and four patients were diagnosed with attenuated adenomatous polyposis coli (AAPC). Our study began with a non-radioactive protein truncation test (PTT) to screen all patients for protein truncating mutations in mutation cluster region (MCR) of APC. Then, we analyzed all 15 exons of APC in patients negative for PTT test by single-strand conformation polymorphism (SSCP) and DNA sequencing. We identified a C to T substitution at codon 1303 (CAA to TAA) resulting in a stop codon (Q1303X) in a FAP patient. Interestingly, no other nonsense or missense mutation was detected in the remaining subjects. Furthermore, two novel silent polymorphisms, 4509G>A and 5898A>G, were found in our patients. Based on our findings, other genes may have the major causative roles in the etiology of FAP in Iran.

P0570. Cytogenetic Analysis of over 500 Iranian Leukaemic Patients

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We analyzed bone marrow and peripheral blood samples of more than 500 Iranian patients referred from major hematology-oncology centers at Tehran and provincial capitals. They were either suspected of a leukemia condition at presentation or being monitored for their response to medication. Cell culturing and banding (GTG & high resolution) were carried out according to standard protocols. Chromosome analysis was performed following ISCN (1995) guidelines. The patients were divided into six major groupings as far as the leukemia subtypes were concerned: CML, AML, ALL, MDS, lymphoma and "others". With regard to the stage they were broadly categorized into "at presentation" and "under treatments". There were more male patients than females, approximately 1.32:1 ratio. In terms of sample type, most cases had bone marrow aspiration whereas peripheral blood was utilized only in a fraction of cases. The common typical chromosomal abnormalities as well as rare and combined 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.

P0571. Diagnostic and prognostic significance of initial genetic examination in childhood acute lymphoblastic leukaemia: results of a ten-year study in Hungary(1993-2002)

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Population-based national study was started in 1993 in Hungary with the aim of providing genetic diagnosis for all new childhood acute lymphoblastic leukemia cases. We investigated distribution of genetic aberrations, their prognostic role and use of different genetic methods

in childhood ALL.

All ten centres of Hungarian Pediatric Oncology Network and diagnostic laboratories accepted cooperation. In addition to initial routine cytogenetic examination flow cytometric DNA-index determination, FISH (numerical aberrations) and molecular genetic tests (ABL/BCR, MLL/AF4, TEL/AML1 gene rearrangements) were introduced to determine different genetic subgroups of ALL. Event-free and overall survival of patients was analysed by Kaplan-Meier method in genetic subgroups. Multivariate analysis (Cox's regression) was performed taking into account different other prognostic factors (age, sex, major congenital abnormalities, initial WBC, therapy, immunophenotype).

588 new cases of childhood ALL were diagnosed in Hungary 1993-2002. Cytogenetic examination was performed in 537 cases (91%) with success rate of 73%. DNA-index was determined in 265 cases (45%), FISH in 74 (13%), TEL/AML1 RT-PCR in 215 (37%). Taking into account results of all genetic tests we came to genetic diagnosis in 456 cases (78%). Proportion of subgroups with good prognosis was lower than expected: hyperdiploidB 18% (80/456), t(12;21) positive 18% (45/215). Univariate analysis showed significantly better 5-year event-free survival in TEL/AML1 positive (82%) and the hyperdiploidB cases (76%) than those with hypodiploid (54%), pseudodiploid (specific translocations: 48%) or combined aberrations (tetraploid/structural aberrations with TEL/AML1 positivity)(45%). Multivariate analysis identified congenital abnormalities, high initial WBC, delay in therapy and specific translocations as main negative prognostic factors.

P0572. Cowden syndrome may be under-diagnosed in the cancer genetics clinic

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Referrals to the hereditary cancer clinic are usually for a personal or family history of breast/ovarian, colorectal or endocrine neoplasias. Depending on the referral source, in some 30-40% of these families gene analysis is indicated; the majority of patients will be negative for mutations. Among the likely reasons for this low mutation yield is the under-diagnosis of phenotypically-mild cases of "rare syndromes" such as Peutz-Jaegers and Cowden syndromes. We would like to call attention to what we perceive as a high frequency of Cowden syndrome among patients referred for breast/colon cancer; we have seen at least three Cowden families in eighteen months, out of approximately 120 referred for hereditary cancer consultation. Patient 1 is a 58 year-old man with personal history of colon polyps and family history of a brain tumor. A frame-shift mutation in the PTEN gene (872-875insA) was identified. Patient 2 is a healthy 40-year-old man with colon polyps, whose daughter had lipomas and enlarged thyroid. Patient 3 is a 64-year-old female with previous breast and uterine cancer, whose daughter had papillary thyroid cancer. Molecular analysis has also initiated in patients 2 and 3.

In all three cases, the clinical diagnosis was suspected not so much by the proband's phenotype as by the combination of history/physical examination and symptoms present in first degree relatives. All three probands have normal intelligence, and only patient 1 has obvious skin lesions. The proband or family's clinical management has been positively impacted by knowledge of the diagnosis of Cowden syndrome.

P0573. The Expression of IMUP-1 and IMUP-2 in Breast carcinoma.

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Immortalization-upregulated protein-1 and 2 (IMUP-1 and IMUP-2) were more frequently expressed in Breast tumor tissues in the same pattern with the gene. Our last data showed the open reading frame of IMUP-1 spans 321bp, coding for a 10.9kDa protein of 106 amino acids, while an insertion of 59bp in the otherwise identical mRNA of IMUP-2 leads to a frameshift, resulting in an 8.5kDa protein of 85 amino acids. We also studied the in vitro proliferation of cells overexpressing IMUP-1 and IMUP-2 in the low serum. Overexpression of IMUPs increased

growth rates and reduced serum requirements for growth compared with control cells. Consistent with these findings, cells overexpressing IMUP-1 and IMUP-2 showed increased IMUPs expression displayed a shortening of cell cycle by FACS cell-cycle distribution analysis. Especially, the level of S phase and G2/M in IMUP-1 and IMUP-2 transfectants cells are increased than cells transfected vector alone. In the yeast two-hybrid system to find the function, IMUP-1 and IMUP-2 was fused with the DNA-binding domain(DBD) of GAL4 and HeLa cell cDNA library was fused with the transcription activation domain(AD) of GAL4. We performed a high-stringency scale procedure to screen IMUP-1 and IMUP-2 against HeLa cell cDNA library and characterized positives by sequence analysis. As the result, 12 novel and 27 known proteins were found as the binding proteins. Some of them are under-checking to confirm whether binding with IMUP-1 and IMUP-2 or not by immuno-precipitation *in vivo*.

P0574. Real-time quantitative polymerase chain reaction for evaluation of MRP1 mRNA level in Iranian AML patients

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Multidrug resistance (MDR) is a complex phenomenon that includes the expression of many different genes regulating drug transport or metabolism, cellular repair or detoxification mechanisms. The expression or co-expression of gene(s) such as multidrug resistance (MDR1) and multidrug associated protein (MRP1) could be at the basis of the resistant phenotype *in vivo*. We developed a quantitative reverse transcription (RT)-PCR method for MRP1 transcripts to evaluate drug resistance, and applied it to clinical samples.

The cutoffs for copy numbers of MRP1 transcripts were defined based on copy numbers in healthy blood. To confirm that the cutoffs reflected biological resistance, we used HL60 (known to have over-expression of MRP1) to examine the correlation between the copy numbers of these transcripts and the biological resistance of this cell line.

After optimizing the assay, it has been employed to test possible prognostic roles of the expression of MRP1 in 30 patients affected by acute myeloid leukemia (AML). Among those patients, 33% showed over-expression of MRP1. These patients are under monitor to test whether or not the high level of MRP1 will contribute to the remission rate and remission periods. These results may also help the prediction of clinical drug resistance in AML Iranian patients.

P0575. Expression of ras p21 oncogene and K-ras point mutations in colorectal cancer

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Mutational activation and overexpression of the ras family genes especially K-ras, have been associated with colorectal tumorigenesis. We investigated the clinicopathological characteristics and point mutation of K-ras oncogene codon 12/13 and ras p21 expression using paraffin embedded materials from 53 colorectal cancer cases and their surrounding normal tissues. Point mutation of K-ras was analysed by PCR-SSCP and followed by DNA sequencing, ras p21 expression was examined immunohistochemically. Mutations of K-ras and overexpression of the ras p21 were detected in 11% and 76% of the tumors, respectively. Ras p21 overexpression did not correlate with any of the clinicopathological parameters examined. K-ras gene mutations were found mostly in presence of a mucinous component within the tumor ($p=0.076$). Follow-up data were available for 43 patients. There was no statistically significant correlation between these alterations and patient survival. Our data suggest that apart from K-ras codon 12/13 point mutations, overexpression of the ras family genes is important in the development of the disease. Furthermore, mucinous secretion in the colorectum may represent a distinct genetic pattern

P0576. Preliminary genetic investigation of the patient with atypical long term malignant melanoma process.

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Malignant melanoma is the most aggressive skin cancer, with incidence rapidly increasing throughout the world at a faster rate than any other tumor type and is usually connected with poor prognosis. We report a case of a melanoma patient X with 46-years long oncological history, which due to such long term of the disease seems to be a medical phenomenon. The patient X was diagnosed first time in 1957 for Ca. basocellulare, and until 2003 recurrences of some other histological subtypes of the disease (i.e. Mel. malignum, Ca. plano keratodes) to different parts of the skin were observed. Moreover, during this period patient X underwent initially 3 radiotherapies, and then 30 surgeries, and survived until today.

In order to explain the basis of this phenomenon, polymorphisms of some genes (CYP2D6, CYP2C9, CYP2C19) were analyzed by TaqMan technology, and DNA repair capacity of peripheral blood lymphocytes irradiated *in vitro* with 2 Gy of γ -radiation was tested by comet assay. Patients X was indicated as a heterozygote for all obtained genotypes. The comparison of DNA repair capacity of patient X, two other melanoma patients and controls has shown decrease for cancer patients. Although the initial DNA damage induced by radiation in cells from patient X was high, the kinetic of strand breaks rejoicing during the 180 min of incubation was faster than those present in other melanoma patients. In conclusion this study requires the further evaluation in order to find for patient X the molecular factors ensuring the better DNA repair capacity.

P0577. Tissue microarray analysis of c-myc copy number alterations in a large number ovarian cancers

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The genetic changes involved in the pathogenesis of ovarian carcinoma are not completely understood. Summarized data of different CGH studies in this tumor type showed that chromosome segment 8q24.1 is consistently gained in more than one third of ovarian cancers. A target gene for amplification of 8q24.1 is likely to be the MYC oncogene, which is located at this sub-band. There is not complete information about the significance of c-myc copy number increases for the disease outcome. Here we have used the high-throughput technique of tissue microarrays (TMA) in order to analyze by FISH 503 ovarian cancers (OC) for the correlation of c-myc copy number changes with clinicopathological parameters - histological type, stage, grade. C-myc was amplified in 35,9 % of OC and gained in 3,8 % of them. We have found that the amplification tends to be more frequent in late-stage (42,5%) vs early-stage (31%) cancers without reaching statistical significance. Regarding histological grade, there was no significant difference between the frequency of amplification in different grade tumors (G1-30%, G2-36%, G3-42,5%). Gains of c-myc did not show an association with clinicopathological parameters. Concerning histological type, the highest frequency of c-myc amplification was established in mixed Mulerian carcinomas - 53%; We concluded that c-myc amplification is common event in ovarian carcinogenesis. It could be targeted in novel anticancer strategies for improving the outcome of the disease.

P0578. Role of the -675 4G/5G PAI-1 gene polymorphism in breast cancer

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The molecular components of Plasminogen Activator system, Urokinase-type Plasminogen Activator (uPA) and its inhibitor PAI-1, are involved in tumor progression and have prognostic value in early breast cancer (BC) patients. The -675 4G/5G polymorphism of the PAI-1 promoter gene is associated with different activity levels of PAI-1 in serum. Cyclooxygenase-2 (COX-2) expression is associated with poor prognosis in early BC. We aimed at studying the -675 4G/5G PAI-1

polymorphism together with the immunohistochemical expression of PAI-1 and COX-2 in BC patients.

Methods: Blood samples from a consecutive series of 199 BC patients and 132 controls were collected and PAI-1 -675 4G/5G polymorphism was analyzed by PCR technique. Immunohistochemical expression of PAI-1, COX-2 and MIB-1/Ki-67 was evaluated in the tissue sample of primitive BC. A score combining intensity and percentage of cellular immunoreactivity, was used to assess the staining of PAI-1 and COX2. PAI-1 positivity was evaluated both in tumor cells and stromal fibroblasts.

Results: No statistically-significant difference of genotypic and allelic frequencies was found between BC patients and controls. The 4G allele was associated with regional lymph node involvement ($P=.04$). No statistically significant association was found between genotype and immunohistochemical expression of the PAI-1 gene PAI-1 score directly correlated with COX-2 expression ($P=.02$), and strong PAI-1 immunostaining was associated with moderate/strong expression of COX-2 ($P=.006$). Tumors with PAI-1 positive fibroblasts showed higher MIB-1/Ki-67 expression ($P=.02$).

These findings suggest that BC patients carrying the 4G allele of the PAI-1 promoter may have an increased probability of axillary lymph node involvement.

P0579. The prognostic relevance of cytogenetic findings in malignant tumours of cervix and ovary

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The knowledge of tumour genetic profile as tumour-related prognostic factor, in correlation with other known predictors ought to led to improved prediction of outcome and individualization of therapeutic strategies.

The aim of the presented study is to estimate the major genetic alterations in examined tumours and to evaluate their significance in correlation with other available markers.

In tumour samples - 46 of ovarian cancer and 20 of cervical cancer tumours histological type and grading were estimated, than immunohistochemical semiquantitative method with quantification of HSCORE according McCarty was applied to determine TP53 and the proliferative marker MIB 1.

Another part of the tissue sample was used for a cytogenetic processing combining conventional method and FISH method using DNA specific probes and painting probes. The isolated DNA samples were preserved to be evaluated by CGH method.

Genetic instability and structural aberrations were detected in 90% ovarian cancer cells and in 48% cervical cells, 10% ovarian tumours and 52% cervical tumours were diploid. Most frequent structural aberrations were unbalanced translocations, deletions and unidentified markers. The rearrangements generally affected chromosomes 1, 2, 3, 4, 8, 9, 11 in ovarian cancer cells and chromosomes 1, 3, 5, 9, 11, 17, 18 in cervical cancer cells.

The wide range of genetic findings corresponds with advanced stage, serous type of poorly differentiated tumours and highly positive TP53 HSCORE.

This work has been supported by the grant of the Ministry of Health of the Czech Republic: IGA-MZ-NH/7659-3: Predictive value of molecular biological parameters in malignant gynaecological tumours.

P0580. Circulating EGFRvIII positive cells in early stage breast cancer: association with factors predicting recurrence

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This work aims to evaluate epidermal growth factor receptor variant III (EGFRvIII) as a possible marker for the diagnosis of breast cancer occult systemic disease.

Thirty three (T1N0M0 or T2N0M0) breast cancer patients, with tumours < 3 cm of diameter, selected for curative surgery and 40 control women

were studied. A RT-nested PCR assay amplifying both EGFR and EGFRvIII was performed.

The RT-nested PCR allowed the detection of 0.5 pg of total RNA from an EGFRvIII expressing cell-line, diluted in 5 µg of total RNA from an hematogenous, non expressing cell-line. EGFRvIII RNA was detected in the peripheral blood of 30% of patients and in none of the control women. All positive results were obtained in the nested PCR (sensitivity of 10-7). When EGFRvIII positive results were compared with the presence of known recurrence factors, no correlation was noticed with microscopic lymphatic node metastases or tumour diameter. There was an association with risk factors of tumour aggressiveness such as absence of estrogen receptors, presence of ERBB2 or histopathologic grades G2/G3. All EGFRvIII positive patients had one or more of these risk factors, whereas in the EGFRvIII negative group of patients, only 56.5% did so ($p<0.05$), supporting EGFRvIII predictive value as a dissemination marker.

Further studies are needed to confirm the role of EGFRvIII RNA detection in the peripheral blood of early stage breast cancer patients as an independent relapse predicting factor.

P0581. Molecular genetic findings in patients with multiple polyposis coli

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Multiple colorectal polyposis (the occurrence of 3 to 100 polyps) might be associated with germline mutations in *APC*, *MYH* or mismatch repair genes.

Patients and methods: A patient (age 62 years) with about 30 colorectal polyps and synchronous occurrence of colorectal cancer and a patient (age 52 years) with tens of polyps have been referred to mutational analysis of the *APC* and *MYH* genes.

Results: In the 1st patient, a novel mutation was found in exon 2 of the *APC* gene located at splice site: c.220G→T, p.Glu74X; IVS2 ds-1. The mutation segregated with the findings of polyposis in presymptomatically tested members of the family older than 50 years. No polyposis was found in mutation carriers younger than 40 years. Methylene tetrahydrofolate reductase (*MTHFR*) gene polymorphism C677T, described to decrease the risk of colorectal cancer, was found in the family. In the 2nd patient, no mutation in the *APC* gene was found, but two most common mutations in the *MYH* gene Y165C and G382D were detected.

Conclusion: We report a novel mutation in exon 2 of the *APC* gene, which segregates with clinical findings in multiple members of the family. Supported by IGA MZ CR NR8103-3/2004.

P0582. Analysis of genotype-phenotype correlation in familial breast/ovarian cancer with *BRCA1* gene mutations in Russia

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BRCA1 mutations may confer as breast so ovarian cancer. One of the conceptions on a decision of the genotype-phenotype correlation problem consists in supposition of influence of a mutation disposition in the gene on breast/ovarian cancer risk.

The *BRCA1* mutation frequency in our sample of ovarian cancer families was 86%. This value was not significant different from *BRCA1* mutation frequency in the families with both breast and ovarian cancer (61%). In families of both types - ovarian cancer only and breast/ovarian - one mutation 5382insC was predominant (50% and 90%, respectively). We compare our proportion of families with breast/ovarian cancer and breast cancer only with published data on samples from the other populations and no found significant difference, in spite of different mutation spectrums. As the results shown, the other genome peculiarities than a mutation position may have influence on cancer localization. Analysis of splitting on breast and ovarian cancer segregation in the families gives a possibility to propose that cancer localization may be defined by interconnection of *BRCA1* gene with

the other gene. We studied some variants of BRCA2 as a candidate for a risk modifier gene under BRCA1 mutations. The evidence for influence on ovarian cancer risk increasing of BRCA1 variant 1038G and BRCA2 variant 372H combination was received (OR = 6,00; P = 0,053).

P0583. No alteration of TH01 DNA marker at patients with skin cancers in Romanian population

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The aim of this study is to determine if the TH01 STR marker suffer any type of alteration (like MSI (microsatellite e instability) or/and LOH (loss of heterozygosity)) in a Romanian population affected by skin cancers. We based our project on the facts that LOH was observed for TH01marker in malign transformations, especially in gastric cancers. For this research we determined (by PAGE technique) the genetic profile of 200 healthy, unrelated individuals and 25 individuals affected by skin cancers from Romanian territory. The allele and heterozygosity frequencies were analysed using population genetic tests (like χ^2 test and Hardy - Weinberg equilibrium) and programs (like AMOVA program).

We concluded that in the Romanian population affected by skin cancers that we have studied, the TH01 marker is not affected by microsatellite instability (MSI) or by loss of heterozygosity (LOH), when compared with the normal, healthy Romanian population.

P0584. STR typing of cancerous tissues for identification purpose

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Malignant tissue samples can be a source of biological material not only for forensic caseworks as personal identification or paternity testing when no other specimen is available, but also for the attribution of a tissue to a patient or in transplantation matter. Aberrations in coding or non-coding region of genome have been reported in cancerous tissues also for those short tandem repeats (STRs) widely used in forensic analysis. Chromosomal instability, loss of heterozygosity (LOH) and microsatellite instability (MSI), were previously described in different kinds of tumors with variable frequency. To increase the number of cancerous samples analyzed by the Identifiler PCR amplification kit, routinely used in forensic laboratories, we present preliminary results on 23 gastrointestinal and 14 urogenital carcinomas with corresponding control normal samples. In our tumor samples we found four types of profile alteration: MSI low, MSI high, partial or complete loss of one allele (pLOH or LOH). pLOH was the most frequent in all the two kinds of cancer. In addition on the samples showing a lower Y-amelogenin signal, no genetic alteration of 11 Y-STR markers profile was detected. Previous microscopic analysis of the tissue sample and careful interpretation of DNA profile in MSI and LOH are required for identification purpose.

P0585. New APC and MYH gene variants among patients with familial adenomatous polyposis in Russia

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The new protein truncating mutations were near 50% of the APC gene mutation spectrum. Deletions were the more frequent both among new mutations and in the whole spectrum (60%). New (unique) deletions in 90% of cases were deletions of 1 or 2 nucleotides while all known (repeated) deletions were deletions of 4 or 5 nucleotides (P=0,0008). The published data on six countries have been analyzed for comparison. The significant difference of unique and repeated deletions found by us was shown for a number but not all of the countries. The selections of patients with FAP from various populations are different on this parameter. Also there were three new missense variants (T1556S, P1780S, T2160A). These variants were not observed

in a combination with protein truncating mutation and were not found in 100 control samples. The results suggest that these variants may be mutations. In contrast, the variant S2621C in two cases of three was found in a combination with a protein truncating mutation and in control samples. The samples of patients without APC mutations were tested on mutations in gene MYH. In four cases the mutations were revealed. There were two cases with mutations on both chromosomes (Y165C/ Y165C and 1395delGGA/R95W) and two cases with heterozygote mutations (Y165C and G382D). The mutation R95W and variant IVS8+21C/A (in combination with G382D) were revealed for the first case.

P0586. IL1 β -511 promoter polymorphism and GEP-NET

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Neuroendocrine tumors of the gut and pancreas (GEP-NET) produce biogenic amines, peptides and chromogranins, especially chromogranine A (CgA). Increased IL1 levels have been described in patients with NET, suggesting a regulatory link with this inflammatory cytokine and its role in tumor growth and morphogenesis.

IL1 β gene is mapped to chromosome 2q14 and a few polymorphisms associated with altered IL1 β production have been described. The aim of our study was to estimate allelic frequency for -511 promoter SNP in IL1 β gene in patients with GEP-NET. DNAs obtained from 30 GEP-NET patients and 150 unrelated healthy volunteers were genotyped for the IL1 β -511 SNP using real-time PCR TaqMan[®] SNP genotyping assay. There was statistically significant difference in the frequency of -511T allele associated with altered IL1 β production between healthy population and patients with GEP-NET. The association between other IL1 β polymorphisms and GEP-NET tumors has to be investigated in the future studies.

P0587. SULT1A1 213Arg is a risk factor for the development of breast cancer in Macedonian woman

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Sulfotransferase A1 (SULT1A1) is an enzyme involved in both detoxification and bioactivation of endogenous and exogenous compounds. A genetic polymorphism in the coding region of the SULT1A1 gene (Arg213His, CGC-CAC) has been associated with modular phenotype, where individuals with Arg allele have a substantially higher enzyme activity. Several studies investigated the influence of this variant on susceptibility of breast cancer (BC), but no definitive conclusion has been obtained. We determined the allele frequency and genotype distributions of SULT1A1 Arg213His variant in 100 sporadic, non-syndromic, breast cancer patients and 200 normal controls from the Republic of Macedonia. The Arg allele was more common in cases than controls (74% vs. 63%, respectively, p=0.0051, RR 1.99; 95%CI:1.0449<R.R<3.745). A dose dependant effect of Arg 213 allele on BC risk was detected (Arg/Arg vs. Arg/His + His/His p=0.036, RR 1.38, 95% CI: 1.0188<R.R.<1.876; Arg/Arg + Arg/His vs. His/His p=0.0178, RR 1.99, 95%CI 1.0449<R.R.<3.7745). When we examined the data by stadium of diagnosis, patients with an advanced disease (stadium III & IV) have a significantly higher frequency of Arg than His allele (82% vs. 18%, respectively, p = 0.0131, RR 0.68; 95%CI 0.5196<R.R<0.9003). No association was found in allelic frequency and genotype distribution of this variant with other clinicopathological parameters of patients (ethnicity, gender, age, menarche). Our data indicate that the SULT1A1 213Arg allele is one of the low penetrance alleles responsible for the development of BC in Macedonian women.

P0588. Homozygous or combined deletion 13q14.3 in patients with B-cell chronic lymphocytic leukemia

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B-cell chronic lymphocytic 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 lymphocytic, found in approximately 50% of patients with B-CLL and suggesting the presence of tumor 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.

FISH analysis, with specific probes for chromosomes 11 (LSI ATM), 12 (CEP12), 13 (LSI D13S319/13q34) and 17 (LSI p53), were performed on 190 samples from B-CLL patients. The most frequent abnormality, as was expected, was del(13)(q14.3) in 50% of cases as sole anomaly plus 4.7% among others. In about 23% of these cases the deletion was found in homozygous or combined form. The frequency of the other aberrations were 11% for trisomy 12, 5.3% for the deletion of ATM and 3.7% for the deletion of p53.

Deletion 13q14.3, as sole anomaly, has a good prognosis in clinical evolution. The implication of homozygous or combined deletion in hematologic features and clinical follow-up of these B-CLL patients will be discussed.

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P0589. RNASEL: a susceptibility gene for prostate cancer in the Jewish population?

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Prostate cancer (PC) is the most commonly diagnosed malignancy in men in the western world, including Jewish men in Israel, and one of the leading causes of cancer-related death. A positive family history is the strongest risk factor for PC and twin studies estimated that 42% of PC risk may be attributed to heritable factors. Highly penetrant susceptibility genes are predicted to account for about 9% of PC cases diagnosed through age 85 and 43% of early onset disease (less than 55 years). Genome-wide linkage scans in hereditary prostate cancer (HPC) families have revealed multiple loci possibly harboring candidate susceptibility genes, although confirmation studies regarding most loci have been inconsistent. *RNASEL/HPC1*, localized at 1q24-25 is a candidate prostate cancer susceptibility gene that has been studied extensively in various patient populations worldwide. We identified the 471delAAAG frameshift mutation in *RNASEL*, the first Ashkenazi founder mutation in a known hereditary prostate cancer candidate gene. We reassessed the frequency of this nonsense mutation in prostate cancer patients, and in patients with breast/ovarian, bladder and colon cancer, and in average risk control population (968 patients and 510 controls, 2.5% and 2%, respectively). Screening the entire *RNASEL* gene, we detected another novel *RNASEL* splice site mutation in 1/121 PC patient using DHPLC, but no deletions were detected, using MLPA, in 300 PC patients. Our results suggest that while *RNASEL* might represent a prostate cancer susceptibility gene, *RNASEL* variations identified to date may account for only a limited number of PC cases in Ashkenazi men.

P0590. Large genomic rearrangements in *BRCA1* and *BRCA2* genes in Spanish breast/ovarian cancer families

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Background: Germ-line mutations in *BRCA1* and *BRCA2* cause a substantial proportion of inherited breast and ovarian cancer. Most of the mutations described are nonsense, frameshift and splice site alterations. Missense mutations have also been detected, their pathological significance being unclear. The frequency of large rearrangements in both genes is not well documented. We screened these rearrangements using the MLPA method (multiplex ligation-dependent probe amplification) in order to estimate their contribution

to breast/ovarian cancer predisposition in Spanish families.

Participants and methods: The MLPA technique was performed in 105 families for *BRCA1* gene and 54 families for *BRCA2* gene by using MRC-Holland kits. No mutation had been found in these families in earlier analyses by different techniques (SSCP, PTT, dHPLC and sequencing).

Results: Nine copy number variants were found within *BRCA1* and two within *BRCA2*. One *BRCA1* alteration involved the deletion of exons 1 to 23 and segregated with cancer in the family. Sequencing of genomic DNA with an apparent deletion of *BRCA1* exon 18 revealed a missense variant in the hybridisation sequence of the MLPA probe. The remaining 7 aberrations are currently being confirmed. Of the families analysed for *BRCA2* a deletion of exons 15-16 has been confirmed to date.

Conclusions: These preliminary results suggest that rearrangements within the *BRCA1* and *BRCA2* genes are not frequent in the Spanish population. MLPA is a sensitive and rapid method for molecular detection of large deletions and duplications in *BRCA1* and *BRCA2* in high-risk breast/ovarian families and can be readily adopted by diagnostic services.

P0591. Cytogenetic and molecular cytogenetic study of 72 CML patients treated with imatinib.

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Additional chromosomal abnormalities in chronic myeloid leukemia (CML) were reported to occur in chronic and accelerated phases of the disease, and also during treatment with interferon and, more recently, with imatinib. The aim of our study was to determine frequency and clinical importance of additional chromosomal changes in 72 patients treated with imatinib.

At the initiation of imatinib treatment, 51 patients had only Ph chromosome, whereas Ph chromosome with additional changes was observed in 21 patients. The most frequent additional changes were der(9) deletion in 8 patients, trisomy 8 in three patients and double Ph chromosome in two patients. The remaining 9 patients displayed different aberrations. After 12 month of treatment the cytogenetic and molecular cytogenetic response to imatinib was analyzed. Eleven patients died within the first year of treatment. The cytogenetic and/or molecular cytogenetic response were presented in 51 (84%) patients (complete response in 7, major response in 41, minor response in 3), 10 (16%) patients were non-responders. All patients with der(9) deletion (n = 8) have responded to the treatment, one has achieved a complete cytogenetic response. Four patients developed new chromosomal aberrations, two of them in the Ph-positive clone.

The results of cytogenetic and molecular cytogenetic study in 72 CML patients after 12 months of imatinib therapy revealed 84% of responders including the patients with der(9) deletion. It confirms efficacy of imatinib treatment and ability to overcome the adverse prognostic significance of der(9) deletions in CML patients.

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P0592. Non Hodgkin lymphoma: Incidence of *Bcl2/IgH* rearrangement in Mexican patients and its relevance as marker for minimal residual disease.

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In the follicular lymphoma present the t(14;18)(q32;q21) which joints one of the J_H segments of the heavy chains of immunoglobulins (IgH) in 14q32 with the *Bcl2* gene in 18q2. **Objective:** To determine the incidence of the *Bcl2*-IgH rearrangement in Mexican patients with follicular lymphoma and with NHL of intermedium and high grade.

Methods: 300 patients were evaluated 155 had low grade lymphoma, 115 intermedium to high grade and 27 the grade was not determined. The analysis was made in blood samples (BS) in 64 cases or bone marrow (BM) in 228. The *Bcl2*-IgH rearrangement was amplified by PCR using primers for J_H and exon-intron 3 region of *Bcl2* (MBR and

MCR regions). The *Bcl-2/IgH* rearrangement was used as marker to determine minimal residual disease stage in cases ranging from 8 to 35 months of evolution. The incidence and tissue type analysis was compared using chi-square statistics. **Results:** In the low grade follicular NHL the *Bcl-2/IgH* was positive in 79% with a breakage in the MBR region in 115 cases and in MCR in 5. In the intermedium to high grade NHL cases it was present in 33% with a breakage in 35 cases in MBR y 2 in MCR. Regarding the tissue type it was positive in 86% of cases in BM and in 42% in BS, with $p<0.0001$. The frequency of *Bcl-2/IgH* rearrangement in Mexican patients was high, highlighting the relevance of the molecular analysis in the diagnosis and its use as a marker for minimal residual disease cases.

P0593. NBS1 germline mutations in children with acute lymphoblastic leukemia

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Alternations of the *NBS1* gene are associated with Nijmegen breakage syndrome (NBS). NBS is an inherited chromosomal instability disorder which is characterized by immunodeficiency and increased predisposition to lymphoid malignancies. Nibrin, the product of *NBS1* gene, is functionally involved in double strand DNA break repair system. Heterozygous carriers of the *NBS1* Slavic mutation have an increased risk of malignant tumors including ovarian, familial breast and prostate cancer. So far reports on *NBS1* mutations in lymphoproliferative diseases are controversial. The aim of the present study was to analyze the mutations in all 16 exons of the *NBS1* gene in children with acute lymphoblastic leukemia (ALL). To discriminate between germinal and somatic mutations DNA was isolated from either leukemic or normal oral epithelium cells. All samples were analyzed by PCR-single strand conformation polymorphism and identified by direct sequencing. The five *NBS1* heterozygous mutations were detected in 9 of 114 ALL cases and only 2 in 160 control individuals (OR=6.771; $p=0.0094$). The most frequent mutation was I171V in exon 5 (5/114 cases versus 1/160 in controls). Mutations D95N, R215W, V210F and 657del5 were observed in single individual. In addition we identified 16 rare sequence variants: IVS7-18G/A, IVS7-29C/T, IVS15+88G/C. The germline origin of the observed mutations was confirmed in all cases. The obtained results suggest that *NBS1* heterozygous germline mutations can be considered as a risk factor in the development of acute lymphoblastic leukemia.

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P0594. Trisomy 19 in a case of BCR/ABL negative chronic myeloproliferative disorder

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In this paper we report the case of a 52 year-old patient with a BCR/ABL negative chronic myeloproliferative disorder and trisomy 19 as a sole chromosomal anomaly, at diagnosis.

The patient has been referred for cytogenetic investigation as a step of the diagnosis algorithm. The hematologic investigation revealed peripheral blood leukocytosis and bone marrow hypercellularity (granulocyte and erythroid series), with no significant reticulin fibrosis. Both classical and molecular cytogenetics methods were applied. Chromosomal studies were performed on GTG-banded slides obtained from bone marrow direct preparation and short term cultures (24h and 48h). FISH with Vysis dual colour, dual fusion BCR/ABL probes was applied on fixed cytogenetic slides.

90% of bone marrow cells were hyperdiploid with trisomy 19 as sole chromosomal abnormality (karyotype: 47,XY,+19). No numerical or structural anomalies were identified in the remaining 10% of the metaphases.

So far, trisomy 19 as a sole chromosomal abnormality has been described by various authors in myeloid malignancies, particularly in acute myeloid leukemia and myelodysplastic syndrome. As a part of complex karyotypes, trisomy 19 has been encountered in chronic myeloid disorders such as idiopathic myelofibrosis and polycythemia

vera. Also, almost 15% of chronic myeloid leukaemia patients gain trisomy 19 as an additional cytogenetic anomaly.

In our case FISH analysis did not reveal cryptic BCR/ABL fusion. Thus, the cytogenetic investigation proves once again, its value for diagnosis and classification of chronic myeloid disorders.

P0595. Tobacco carcinogen metabolism genes, tobacco smoking and gastric cancer risk in the European population

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Gastric cancer (GC) results from a multi-stage carcinogenic process in which interactions between environmental and genetic factors play an important role. This study was aimed at analysing the association between GC, tobacco smoking and polymorphisms in the CYP1A1 (T461N, I462V and 3801T>C), CYP1A2 -154A>C, NAT2 (R64Q, G286E, R197Q and 481C>T) and EPHX1 (Y113H) genes involved in carcinogen metabolism. The study includes 278 GC cases and 1101 paired controls from the Eur-Gast case-control study nested within the EPIC cohort. Genotyping was performed by real-time PCR and probe melting curve analysis in a LightCycler. Association was analysed by conditional logistic regression models. Interaction was analysed by the likelihood ratio test (LRT). Analysis of smoking habits revealed that 65% of cases and 55% of controls were ever smokers (OR=1.64 IC 95%: 1.22-2.33). Polymorphism analysis revealed that the CC genotype of the exon 3-28T>C (Y113H) SNP in EPHX1 was a risk factor for GC in univariate codominant (OR=1.72, IC:1.07-2.77) and recessive (OR=1.61, IC:1.02-2.53) models. Regarding interaction, tobacco smoking did not modify the risk associated with the EPHX1 variant. However, NAT2 G286E and CYP1A1 I462V were risk factors only in smokers (OR =2.88, IC: 1.38-6.03, LRT for interaction $p=0.01$, and OR=3.05, IC: 1.69-5.50, LRT $p=0.08$, respectively for smokers carrying the NAT2 and the CYP1A1 variants vs. wild type never smokers). These results are the first to suggest a positive interaction between tobacco smoking and the NAT2 G286E and the CYP1A1 I462V polymorphisms in gastric carcinogenesis.

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P0596. Cytogenetic and molecular cytogenetic characterization of a Endometrial Stromal Sarcoma presenting a t(10;17)(q22;p13) translocation.

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Endometrial stromal sarcomas (ESS) are rare neoplasms with variable clinical presentation, as a common feature local myometrial invasion is combined with extrauterine metastases. Cytogenetic aberrations have been described in 30 cases, they showed a broad variety of chromosomal aberrations with non-random involvement of chromosome 6, 7 and 17.

The most common translocation of this tumor seems to be a t(7;17)(q15-q21;q12-q21) generating a JAZF/JJAZ1 fusion gene. A second translocation, a t(10;17)(q22;p13) has been described in two of the 30 ESS cases.

We report on a female patient, age 59 years at time of diagnosis of a metastasing ESS. In addition the family history of the patient was positive for breast cancer. Cytogenetic evaluation of the tumor revealed a complex karyotype. Multicolour FISH was performed and combined analysis of G-banded and M-FISH metaphases revealed a reciprocal t(10;17)(q22;p13) and a t(12;13)(q24;q14).

Therefore the t(10;17)(q22;p13) is a recurrent translocation in ESS and because it is an uncommon translocation in other neoplasms, it seems to be a ESS specific aberration.

A detailed breakpoint characterization using FISH with band specific BAC clones is performed.

P0597. Hypermethylation of the Fanconi Anemia gene FANCF in bladder carcinoma

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Bladder carcinomas frequently show extensive deletions on 9p and/or 9q, potentially including the loci of the Fanconi anemia (FA) genes FANCG and FANCC. Using FANCD2 immunoblotting, the proficiency of the FA/BRCA pathway of 22 established bladder carcinoma lines was tested. A single cell line proved defective for FANCD2 monoubiquination and particularly hypersensitive towards mitomycin C. Exploiting this sensitivity for functional complementation analysis after transduction of BFTC 909 with retroviral vectors (separately expressing six FA genes of the nuclear core complex), MMC sensitivity of the line was restored to normal specifically with the FANCF vector, not by FANCC or FANCG as originally expected. M-FISH revealed the presence of two chromosomes 11 in this hypo-tetraploid line (DNA index 1.75). CGH did not suggest loss of heterozygosity of 11p15 and sequencing of the FANCF gene of BFTC 909 failed to identify mutations. Methylation of cytosine residues in the FANCF promotor region and beyond was indicated by methylation-sensitive HpaII-restriction assay and bisulfite DNA modification. There was no evidence for FANCF promotor hypermethylation in surgical specimens of 10 bladder carcinomas. FANCF silencing has also been observed irregularly in malignancies such as AML and carcinomas of the pancreas, cervix, ovary and prostate. Epigenetic interruption of the FA/BRCA pathway and/or silencing of other genes in the area of 11p13-11p15, reportedly a hot spot of methylation, constitutes a cellular response at unclear genetic end in a subset of human tumors.

P0598. NBS1 germline mutations in a series of 550 Polish pediatric patients with sporadic hematological malignancies

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Nijmegen breakage syndrome (NBS) is a human autosomal recessive disease with defects in DNA repair, which is characterized by immune dysfunction, radiation sensitivity, and a strongly increased risk of cancer, in particular of lymphoid origin. To examine the role of the Slavic founder mutation 657del5 and the R215W variant allele in constitutional susceptibility to childhood lymphoma and leukemia, we screened by PCR-SSCP analysis remission DNA samples from 550 children treated at 13 Hematology and Oncology Centers making up the PPSSG. Frequencies of heterozygosity for the mutations 657del5 and R215W in the control group, matched by place of patient residence, were calculated as 0.0060 and 0.0027, respectively. We found 5 carriers of the 657del5 mutation among the patients: 3/272 patients with childhood acute lymphoblastic leukemia (ALL) and 2/215 children and adolescents with non-Hodgkin lymphoma (NHL); no carrier was found among 63 patients with Hodgkin lymphoma (HL). R215W was not detected in any studied group. The patient group is characterized by an increased odds ratio (range from 1.48 to 1.85 with 95% CI) for the occurrence of mutation 657del5 in comparison with the total Polish population; the Yates corrected chi-square test confirmed that the occurrence of mutation 657del5 is indeed higher in patients with ALL and NHL ($p < 0.05$). Nonetheless, NBS1 gene heterozygosity is not a major risk factor for lymphoid malignancies in childhood and adolescence.

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P0599. Cytogenetic and molecular characterization of t(4;12) involving the ETV6 gene with a novel translocation partner on chromosome 4 in a case of acute myeloid leukemia.

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We report a 55 years old female patient with idiopathic myelofibrosis and systemic mastocytosis, first diagnosed in 2002. Cytogenetic investigation of a bone marrow sample showed a normal karyotype. Transformation to a AML FAB M0 was diagnosed after two years. Cytogenetic analysis revealed following karyotype :46,XX,t(4;12)(q26;p13). Detailed molecular cytogenetic studies confirmed the involvement of ETV6 on 12p13. The ETV6 gene (also known as TEL) is the main target of chromosomal translocations affecting chromosome band 12p13. The rearrangements fuses ETV6 to a wide variety of partner genes in both myeloid and lymphoid malignancies. At present, more than 40 distinct translocations have been described, some of them have also been characterized at the molecular level. As a rare recurrent translocation in acute leukemia t(4;12)(q11-q12;p13) has been characterized at the molecular level, a fusion gene between ETV6 and CHIC2 could be identified. In our case the involvement of ETV6 could already be demonstrated but the breakpoint on chromosome 4 is different (4q26) from the upper described translocation. Therefore a molecular cytogenetic search for the novel translocation partner to ETV6 is performed.

P0600. Genomic deletions in MSH2 or MLH1 genes in patients with Lynch syndrome detected by MLPA

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Hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) is an autosomal dominant condition predisposing to colorectal, endometrial, including other cancers, caused by germline mutations in mismatch repair genes. Successful detection of a pathogenic mutation enables predictive testing in relatives at risk for the disease. While the majority of mutations - nucleotide substitutions, short insertions or deletions - could be detected by standard PCR and sequencing-based screening methods, genomic deletions escape detection when using such protocols. Recently, multiplex ligation-dependent probe amplification (MLPA), has been applied for examination of genomic deletions. Hereby, we studied the type and frequency of genomic rearrangements in MSH2 or MLH1 genes in patients with HNPCC in the Czech Republic. Genomic DNA from 53 patients fulfilling Amsterdam criteria II was investigated. Initially, mutation screening was performed by combination of PCR-amplification of individual exons of the MSH2, MLH1 and MSH6 genes, DGGE and direct sequencing. This screening revealed a pathogenic mutation in 37/53 (70%) of patients (30 in MLH1, 5 in MSH2, and 2 in MSH6 gene). In 16 patients, classified as mutation-negative after the mutation screening, the presence of a gene rearrangement was analysed by MLPA. With this method we identified 3 deletions in the MSH2 gene (exons 1-2, exon 3, exons 9-16) and one deletion in the MLH1 gene (exons 1-13). Genomic rearrangements in either MSH2 or MLH1 genes were found in 4/16 (25%) previously mutation-negative patients and the overall mutation detection rate in our patients reached approximately 77%.

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P0601. A rare chromosomal translocation in a B-CLL patient.

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Chronic lymphocytic leukaemia (CLL) is a disorder of morphologically mature but immunologically less mature lymphocytes and is manifested by progressive accumulation of these cells in the blood, bone marrow and lymphatic tissues. Bone marrow routine cytogenetic analysis of B-CLL usually fails to identify abnormal clones due to a low rate of

spontaneous mitoses, a poor response to mitogen stimulation and/or a bad metaphase quality. Nevertheless recent studies utilising fluorescent *in situ* hybridization (FISH) suggest that prognostically significant chromosomal abnormalities should occur in B-CLL more frequently than previously recognised.

A case of a man, aged 77, affected by B-CLL confirmed by the immunophenotypic profile, not responding to therapy (clorambucil and cortisone), is presented.

Combining both conventional and molecular cytogenetic techniques in bone marrow cells we found a deletion involving the chromosome 13 long arm (13q21-qter) and an additional chromosomal rearrangement, involving chromosomes #2 and #18 (q32;q12).

It is already known that BCL-2 gene is localized in 18q21 band and overexpressed in about 85% of B-CLL tumours without any visible chromosomal rearrangement in that region.

Then in this case we could hypothesize a position effect enhancing the BCL-2 gene expression.

Moreover it could be hypothesized that cryptic rearrangements could occur in those cases with BCL-2 gene overexpression and without any visible chromosomal rearrangements.

A major chromosome 13 deletion (13q14) characterizes about 46% B-CLL cases. Our finding shortens the critic region in agreement with previous evidence showing that the RB gene in 13q14 is not involved in B-CLL.

P0602. Mutation analysis of the BRCT domain proteins MDC1 and 53BP1 in German breast cancer patients

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The Mediator of DNA Damage Control 1 (MDC1) and the p53-Binding Protein 1 (53BP1) are central modulators of ATM signaling after DNA double-strand breaks and share a common motif with BRCA1, the BRCT domain. We thus considered these two genes as candidate breast cancer susceptibility genes and performed a mutation analysis of the whole coding sequence in 24 German patients who had hereditary breast cancer without identified BRCA1 or BRCA2 mutations. Selected mutations were subsequently screened in hospital-based series of 133 patients with bilateral breast cancer, 133 patients with unilateral breast cancer and 133 random individuals. One amino acid substitution of MDC1 was observed more often in bilateral breast cancer than in population controls ($p<0.01$). Our study also uncovered a polyvariant allele of the MDC1 gene, harbouring a 123 bp insertion within the coding region, that occurred at a modestly increased frequency in the breast cancer cohorts compared with the population controls (OR=2.1, 95%CI 0.7-6.3). Immunoblot analyses of lymphoblastoid cell lines from an insertion carrier revealed only subtle changes in the expression and radiation-induced phosphorylation of this variant MDC1 protein. In the 53BP1 gene, one common missense substitution was less abundant in the homozygous state in breast cancer patients than in population controls ($p=0.02$), and a rare two-amino-acid deletion was identified in three breast cancer patients. In summary, while our data do not provide evidence for truncating mutations of MDC1 or 53BP1 in hereditary breast cancer, the association of frame-conserving gene alterations with breast cancer deserves further investigation.

P0603. Gene expression profiling in endometrial cancer

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Endometrial cancer is one of the most common neoplasms of reproductive system. According to data accumulated in last decades it has been concluded that microsatellite instability and PTEN, K-ras, β -catenin gene mutations are crucial for endometrial cancer etiopathogenesis. Moreover, it is well known that all genes encoded proteins involved in cell cycle regulation, cell differentiation or surrounding tissues infiltration are candidate for lesions leading to tumor and metastases development. Complexity of molecular mechanisms leading to endometrial cancer development stress that only experiments based on array technique can help to collect enough data to understand all interactions between different molecular pathways crucial for tumor

formation. Using MacroArray technique we analyzed expression of oncogenes, tumor suppressor genes, cell cycle regulators and others genes encoded proteins involved in signal transduction. Comparison of obtained data with tumor grade allowed us to give shape to gene expression profilings reflecting G2 tumor grade.

P0604. The HPC1/RNASEL 471delAAAG founder mutation in Ashkenazi women with breast/ovarian cancer.

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471delAAAG is a 4-bp deletion mutation recently identified in the HPC1/RNASEL gene as a founder mutation associated with prostate cancer in patients of Ashkenazi descent. The objective of this study was to determine whether this mutation confers increased risk for breast and ovarian cancer in this population. The mutation frequency was thus determined in 260 breast/ovarian cancer patients sub-grouped on account of their being carriers of the BRCA1/2 predominant Ashkenazi mutations (130 BRCA1/2 mutation carriers and 130 non-carriers matched for morbidity and age at disease onset). Asymptomatic women from high-risk families sub-grouped as carriers or non-carriers of BRCA1/2 mutation (n=94) were also tested. Additionally, 200 healthy individuals from the general population were used as a comparison group. PCR products containing the 471delAAAG mutation were analyzed by the WAVE DHPLC apparatus (Transgenomics). All abnormal DHPLC profiles were confirmed by restriction assays designed to differentiate between the wild type and the mutant allele. In all the cohorts studied, only two BRCA1/2 mutation carriers, with breast and ovarian cancer, respectively, were found to carry the 471delAAAG mutation. Our data contradict previous observations as regards the prevalence of the 471 4-bp deletion in the general Ashkenazi population estimated previously at 4%. As such, the 471delAAAG mutation does not seem to contribute to cancer morbidity in this selected population.

P0605. Overexpression of cyclin D2 and D3 genes in human acute leukemia cells.

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The cyclins are considered to be molecules involved in basic pathway of cell division. The overexpression of these positive cell cycle regulators seems to be one of the factors responsible for incorrect regulation of the cell cycle machinery and consequently uncontrolled cell proliferation. The most important cyclins for the G1 phase of cell cycle are D type cyclins. Their level and activity decide about start of cell division.

In our research we examined the level of D type (D1, D2, D3) cyclins genes expression in bone marrow samples obtained from 26 patients with acute myeloblastic leukemia and 19 with acute lymphoblastic leukemia before treatment. The obtained results were compared with peripheral blood lymphocytes. For the assessment of cyclins' mRNA we used semi-quantitative method - The Multi-Probe RNase Protection Assay System (Pharmingen) with hCYC-1 set of probes. Proteins were detected by immunocytochemistry.

The results we obtained shows the leukemic cells reveal significantly high levels of cyclin D2 and D3 mRNA than lymphocytes. The level of cyclin D1 mRNA was similar in leukemic blasts and in lymphocytes. All D type cyclins protein was localized in cell nucleus in all examined cells.

Cyclins D2 and D3 are connected with disturbances of cell differentiation. The increased level of D2 and D3 cyclins expression seems to be concerned with incorrect cell differentiation in acute leukemia. Maybe, the blocking of cyclin D2 and D3 genes expression or protein activity will cause proper cell differentiation.

P0606. Genotyping of CYP3A5 and MDR1 polymorphisms in Bulgarian patients with colorectal cancer

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Single nucleotide polymorphisms (SNPs) may contribute to the cancerogenesis. Genetic variants in xenobiotic metabolising enzymes

and transporters might be responsible for a higher susceptibility to colorectal cancer.

We investigated the frequency of CYP3A5 polymorphisms (CYP3A5*6, CYP3A5*3, CYP3A5*3B and CYP3A5*2) and MDR1 SNPs (3435C>T and 2677G>T) in Bulgarian patients with colorectal cancer. We analysed tumor and normal tissue of 30 patients. The results, presented in table 1, are compared with the results of 36 healthy controls. We found no evidence of somatic mutations in the tumor samples as genotypes always matched with those of the corresponding normal tissue samples. No significant difference was observed in the studied allele frequencies between patients and controls. Enlarged number of patients and controls will be included in further studies.

Table 1. SNPs frequencies in patients with colorectal cancer and controls (allele-1 - wild-type alleles; allele-2 - variant alleles; N=number of studied individuals; n=number of alleles or number of heterozygous, homozygous)

SNPs	Patients (N=30)		Controls (N=36)	
	n	frequency	n	frequency
CYP3A5*6				
Allele-1	60	1	72	1
Genotypes (1/1)	30	1	36	1
CYP3A5*3				
Allele-1	52	0.867	69	0.958
Allele-2	8	0.133	3	0.042
Genotypes (1/1)	22	0.733	33	0.917
(1/2)	8	0.267	3	0.083
CYP3A5*2				
Allele-1	60	1	72	1
Genotypes (1/1)	30	1	36	1
CYP3A5*3B				
Allele-1	60	1	72	1
Genotypes (1/1)	30	1	36	1
MDR(3435C>T)				
Allele-1	31	0.517	41	0.567
Allele-2	29	0.483	31	0.433
Genotypes (1/1)	8	0.267	13	0.361
(1/2)	15	0.500	15	0.417
(2/2)	7	0.233	8	0.222
MDR(2677G>T)				
Allele-1	35	0.583	49	0.681
Allele-2	25	0.417	23	0.319
Genotypes (1/1)	11	0.367	17	0.472
(1/2)	13	0.433	15	0.417
(2/2)	6	0.200	4	0.111

P0607. Human genetic and skin in vitro studies in Gorlin syndrome

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Gorlin syndrome or Nevoid Basal Cell Carcinoma Syndrome (NBCCS) is an autosomal dominant disease characterized by developmental abnormalities and cancer predisposition to basal cell carcinoma (BCC). The NBCCS gene is the PATCHED (PTCH) tumor suppressor gene encoding for the Sonic Hedgehog receptor. While mutations reports and clinical studies have been published, the clear clinical delimitation of the disease and the molecular understanding of the BCC predisposition are missing.

Through a clinical research network, we screened for PTCH mutations

in 135 unrelated NBCCS patients, the largest cohort studied so far. 44 novel mutations were identified. Our results underline the high frequency of de novo PTCH gene mutations, and help us to revisit the clinical diagnosis criteria. However the non-detection of PTCH mutations pinpoint the non-exploration of other genetic events. So new molecular diagnosis strategy based on multiplex genomic PCR has been set up to explore the occurrence of PTCH genomic rearrangement in NBCCS patients. Preliminary results will be presented.

Beside, it is unclear how germinal mutation of PTCH result in the predisposition to BCC in NBCCS patients. To answer the question, we generated an unique collection of NBCCS fibroblast and keratinocyte primary strains from skin of NBCCS patients harboring a known germinal PTCH mutation. Although UV and gamma irradiation have been shown to contribute to BCC in NBCCS patients, NBCCS keratinocytes are neither photosensitive nor radiosensitive. Then, we reconstructed NBCCS skin in vitro. Preliminary results suggest the crucial role of abnormal mesenchymal/epithelial interactions in predisposition of NBCCS patients toward BCC development.

P0608. Investigation of molecular-genetic predictors in selection of personalized therapy for non-small cell lung cancer (NSCLC)

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Lung cancer is serious civilisation disease with high incidence and mortality. Over 6000 new cases are diagnosed in Czech republic each year. Despite to development of new drugs prognosis remains very poor. Most patients are treated by combined chemotherapy, radiotherapy and recently also by targeted biological therapy. The associated expenses are significant, probability of remission is less than 50% and side effects of combined therapy are serious. Recent advances in research offer several genetic markers enabling prediction of therapy response for each individual patient. For example such molecular predictors can be used to indicate targeted biological therapy to spare the patient adverse effects of cytostatics or to avoid ineffective chemotherapy.

Here we report investigation of several molecular predictors for rational selection of treatment for patients diagnosed with non-small cell lung cancer (NSCLC). We present utility of several molecular-genetic markers including somatic mutations in EGFR and k-ras genes from tumor tissue complemented by monitoring of inherited single-nucleotide polymorphisms (SNPs) in ERCC1 and ERCC2/XPD genes from patient's blood samples. The obtained molecular profiles are used to design the most effective combinations for chemotherapy or targeted biological therapy.

P0609. Microsatellite instability in Czech patients with colorectal cancer

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Detection of microsatellite instability (MSI) is a part of mutational analysis of colorectal cancers (CRC). Characteristic phenotypic feature of MSI indicates loss of mismatch repair (MMR) in tumor cells. Loss of MMR can be caused by somatic mutation or epigenetic inactivation of that gene.

We studied MSI in 199 tumors from 142 patients with colorectal cancer. Of these, 35 patients fulfilled Amsterdam criteria (group A+), 68 patients were familial (group A-) and 39 were sporadic cases (group Spor). We used method of fragmentation analysis (ABI Prism 310 Genetic Analyzer) with fluorescent labelled primers; three mononucleotide (BAT-RII, BAT-25, BAT-26) and five dinucleotide (D2S123, D3S1029, D5S346, D17S250, D18S58) repeat loci were analysed. Tumors were classified as MSI-H (high degree of MSI, 2 or more loci with MSI), MSI-L (low degree of MSI, 1 unstable locus) and MSS (stable, no MSI detected). We detected 72 tumors with MSI-H, 10 tumors with MSI-L and 117 MSS tumors. In 41 of MSS tumors LOH was detected.

Results correlated with mutational analysis of MMR genes (hMLH1, h MSH2, hMSH6) and immunohistochemical detection of their proteins. The work was supported by Grant Agency of Charles University (Grant No. 17/2001).

P0610. Genomic instability patterns in patients with sporadic colorectal cancer

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It is generally accepted that colorectal cancer (CRC) can progress through either chromosomal instability (CIN) or microsatellite instability (MSI). We evaluated the pattern of genomic instability in 91 sporadic colorectal cancer patients by analyzing the MSI status and loss of heterozygosity (LOH), as evidence for CIN, in 8 microsatellite markers located on chromosomal arms 2p, 3p, 5q, 17p and 18q. Five of the markers were from the NCI-recommended panel for MSI testing, while the others were located in the regions harboring tumor suppressor (APC, p53) or mismatch repair (MLH1, MSH2) genes important for CRC development. MSI was detected in 11 cancers (12.1%) that were primarily located in the right colon, with mucinous histotype and lower stage at diagnosis. The LOH of at least one chromosomal arm was detected in 57 (62.3%) of cancers, of which the most common was 18q (84.2%) followed by 5p (54.4%) and 17p (45.6%). Four out of 11 patients from the MSI group also exhibited LOH of at least one chromosomal arm indicating a significant overlap between the two types of genomic instabilities. In 27 patients (29.67%) we did didn't detect any evidence of CIN or MSI. Our data indicate a significant overlap between CIN and MSI phenotypes and suggest that in almost 1/3 of all cases the molecular mechanism is not related to either CIN or MSI type of genomic instability.

P0611. Spectrum of chromosomal abnormalities in hematological malignancies

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Malignant disorders are no longer considered as "killer-disease" owing to continuous refinement of cytogenetic and molecular technologies, and development of molecular medicines. Among all neoplastic diseases, hematological malignancies are well characterized with the underlying genetic causes and treatment facilities. Chromosomal/genetic abnormalities are considered important parameters for classification and risk evaluation of myeloproliferative disorders. We present the spectrum of chromosomal abnormalities in different hematological malignancies detected in 75 cases referred for diagnosis. Based on morphology and immunophenotyping, they were classified as acute lymphocytic leukaemia (ALL-12), acute promyelocytic leukaemia (APL-4), chronic myeloid leukaemia (CML-15), multiple myeloma (MM-3), myeloplastic syndrome (MDS-15), Burkitt's leukaemia (BL-2), acute myeloid leukemia (AML-13) and others (13). Normal karyotype was detected in 26 patients, whereas others were detected with numerical or structural abnormalities. CML patients were presented with Philadelphia chromosome, BCR-ABL rearrangements, and even three-way translocations, viz. t(9;22;11) and t(5;9;22). Constitutive abnormality, pericentric inversion in 9, was observed as genetic predisposition of early onset of myelodysplastic syndrome in two cases (16y and 18y), who further acquired deletion in 13q, and der(3),t(10;11),+9 respectively. Inversion in 16 (2), t(15;17) (2) and t(8;21) (4) were recorded in AML and APL patients. MDS patients had a number of deletions and numerical abnormalities and complex rearrangements. Presence or absence of characteristic abnormalities in different patients helped classifying the disorder accurately and treating patients accordingly. In many instances, additional abnormalities directed concomitant therapy for combating the secondary clone. Expression or amplification of different oncogenes, its correlation with clinical presentation and treatment outcome will be presented.

P0612. Tumor characteristics of BRCA 1-associated familial breast cancer from Russian population

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Cancer arising in carriers of mutation in BRCA 1/2 genes differs from sporadic breast cancer of age-matched control.

195 breast or/and ovarian cancer patients with strong cancer history from cancer-genetics registry of Russian N.N.Blochin Cancer Research Center were screened for germline mutation in BRCA 1/2 genes using a CSGE and direct sequencing.

Results: 57 BRCA 1/2 mutation carriers have been detected. High frequency of mutation 5382 insC (76.4% of all revealed mutations) was shown. A multivariate analysis was performed, it includes 32 cases of BRCA 1-linked breast cancer (BC). Control groups consisted of 57 patients with sporadic BC selected on the basis of age and disease stage.

Mean age in the groups studied was 39 years in cancer patients and 41 years in control. Mean menarche age was 13.3 years in BRCA-carriers and 13.7 years in sporadic cancer patients. In 4 patients with pathological BRCA genotype (12.2%) breast cancer developed during pregnancy. Histopathological characteristics of BRCA-associated breast cancer were: (1) infiltrated ductal carcinoma (89.8%/87.7%); (2) high grade - 58.2%/29.2%; (3) prominent lymphocyte infiltrate (60.2%/34.5%); (4) negative receptors of estrogen -77.6%/38.8%; (5) complete clinical response to primary chemotherapy (anthracycline-based treatment) followed by surgery (98.1%/38.2%) compared to the control group (p<0,05).

Mouse mammary tumor virus (MMTV)-related sequences were found by specific PCR in 39% of sporadic BC patients and in 42% familial BC, while these sequences were detected in about 57% of BC patients during pregnancy or shortly after delivery. MMTV-related retroviral agent might be considered as BC risk factor, especially in familial and gestational BC cases.

P0613. RB1 Loss of the heterozygosity analysis in colorectal cancer patients from Singapore

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The RB1 gene is a tumour suppressor gene involved in many important roles in cell division, differentiation and apoptosis in many cell types. Allelic loss of RB1 has been observed in retinoblastoma as well as in a number of other tumours including prostate cancer (53%), bladder cancer (61%), breast cancer (32.5%) and ovarian cancer (61%). We determined the frequency of loss of heterozygosity (LOH) of the RB1 gene in 55 colorectal cancer patients from Singapore. The patients comprised 36 male and 19 female patients with a median age at surgery of 60.1 years. LOH analysis was done using Genescan analysis on the ABI 377 sequencer at three intragenic (D13S153, Rb14 and Rb1.20) and two extragenic (D13S218 and D13S137) microsatellite markers for each sample. LOH at one or more marker was observed in 31% of our patients. No statistically significant differences in occurrence of LOH between males and females or with Dukes' staging of tumours were observed. LOH at RB1 occurred in patients with Dukes' stages A and B, suggesting involvement of this gene in the early stages of colorectal carcinogenesis in these patients.

P0614. Molecular Analysis of Complex BCR-ABL1 Gene Rearrangements in Chronic Myeloid Leukaemia

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Leukaemia often happens when DNA that encodes genes at different chromosomal sites spontaneously recombines in progenitor cells of the haematopoietic system. In one subtype, chronic myeloid leukaemia

(CML), the *BCR-ABL1* fusion protein is responsible for the clinical phenotype, and is expressed after recombination occurs between the *BCR* gene on chromosome 22 and the *ABL1* gene on chromosome 9. In about 90% of CML patients, this recombination results in a cytogenetically visible, simple reciprocal exchange involving the long arms of chromosome 9 and 22. In the remaining cases, recombination between *BCR* and *ABL1* can be more complex involving additional chromosomal sites that may be visible cytogenetically or cryptically concealed within a normal appearing chromosome complement. Previously, we isolated *BCR* gene fragments linked to other participating chromosomes from four patients with complex rearrangements. Unexpectedly, coding regions were found disrupted at the additional chromosome-*BCR* recombination site in two cases. A combination of inverse-PCR and DNA sequence analysis has now been applied to isolate and characterize genomic features at *BCR* recombination sites in a new series of 20 CML patients having complex *BCR-ABL1* rearrangements. By this approach, *BCR* fragments linked to additional participating chromosomes have been isolated from seven further patients. Preliminary sequence analysis shows a disrupted coding region in one case at the *BCR* recombination site and in a further five cases the breakpoints map 95 bp and up to ~25 kb from coding domains. Additional gene involvement has potential to influence the biology and pathology of the leukaemic disease, with further study required to substantiate and extend these novel findings.

P0615. Detection of mitochondrial DNA mutations in gastric adenomas

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Alterations of oxidative phosphorylation in tumor cells are thought to play a role in cancer growth. Recently, a high frequency of mitochondrial DNA (mtDNA) mutations have been reported in human tumours. To determine the frequency and distribution of mitochondrial mutations in gastric adenomas of Italian patients, we screened the displacement-loop (D-loop) region and the hypervariable regions 1 (HV1) and 2 (HV2) of mtDNA. DNA was extracted from 24 samples of matched adenomas and normal tissues embedded in paraffin blocks. We performed the amplification and automated direct sequencing of the following mtDNA regions: L15990-H617; L15990-H16434; L16431-H162; L039-H407; L361- H617.

In 9/24 adenomas, but not in normal tissues, some mtDNA mutations have been identified: heteroplasmic transition G:C/A:T at nucleotide position (np) 16004, heteroplasmic transition C:G/T:A at np16495 (2 cases); a 1-base-pair A:T insertion at np288 (1 case); heteroplasmic C:G/G:C transition at np16168 (1 case); a 1-base-pair C:G insertion at np16244, heteroplasmic C:G/T:A transition at np16495 (1 case); heteroplasmic T:A/G:C transition at np10, heteroplasmic A:T/T:A transition at np202 (1 case); heteroplasmic C:G/G:C transition at np16168 (1 case); homoplasmic C:G transition at np16013, a 1-base-pair A:T insertion at np288 (1 case); heteroplasmic C:G/T:A transition at np16356 (1 case). In our cases, the mtDNA mutations were found only in the gastric adenomas, but not in matched normal tissues. Our findings suggest that mtDNA is mutated in a subset of gastric tumours, and that the disruption of the mtDNA repair system can be involved in gastric adenomas of Italian patients.

P0616. Oncogene identification in Squamous Cell Carcinomas : mapping of chromosome 3 aberrations by array CGH and functional analysis of candidate oncogenes.

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We are interested in mapping chromosomal aberrations in Squamous Cell Carcinomas (SCC) and analyzing their consequences. Indeed,

chromosome 3 aberrations are among the most recurrently observed aberrations in these tumors: deletions of the 3p arm are often found and several tumor suppressor genes have been proposed. Amplifications and/or gains of the 3q arm are present in a majority of tumors and have been linked to a poor prognosis for the HNSCC patients. However, the oncogene targeted by these amplifications/gains has not been isolated yet even if some candidates have been proposed.

To better dissect these aberrations, we have analyzed a series of 30 Lung SCC and 50 Head and Neck SCC (tumors of advanced stages: T3 and T4) by array CGH, using a home-built chromosome 3 dedicated array composed of 307 BAC clones. This screening allowed us to narrow down the common region of amplifications/gains to a small 3q26 genomic segment (< 2 megabases), highly amplified in approximately 20 % of these tumors, and gained in more than 80 %. Bioinformatic analyses show that this consensus region contains only 6 known Refseq genes. To discriminate between these candidate oncogenes, we are currently analyzing their expression levels in SCC with or without 3q26 amplification as well as the functional consequences of their overexpression in cellular models. This work should pinpoint a new major oncogene of the squamous cell carcinogenesis, commonly amplified and overexpressed in consequence.

P0617. Prevalence of the *FMR1* mutation in Taiwan assessed by large-scale screening of newborn boys and analysis of DDX548-FRAXAC1 haplotype

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If carrier women could be identified in time and take appropriate measures, fragile X syndrome can be prevented. Wide screening of women to be or in their early pregnancy was considered a good approach to identify carriers without misdetection. Nevertheless, we argued against the cost-effectiveness of implementing such a screening program in Taiwan, due to the lower carrier rate found in our pilot study. To reliably estimate the prevalence of mutant *FMR1* gene in Taiwan, we anonymously screened 10,046 newborn boys using bloodspot PCR. Among them, the sample from one boy, who was most likely a victim of FXS, failed repeatedly in PCR amplification. The estimated prevalence of premutation (55-200 CGG repeats) and intermediate alleles (45-54 CGG repeats) were 1:1,674 (n=6) and 1:143 (n=70), respectively. All these estimates were constantly lower than that reported in Caucasian populations, with variable statistic significance. Furthermore, when comparing analyses of the distribution of alleles at the two most often investigated microsatellite loci, DDX548 and FRAXAC1, between 100 control and 28 unrelated fragile X chromosomes, we found no apparent founder haplotype prevalent among the fragile X patients. Because a few founder haplotypes were reportedly prevalent in two thirds of fragile X alleles in Caucasians and in Chinese from central China, we thus suggested that lack of founder fragile X chromosomes might result in a relatively low prevalence of mutant *FMR1* gene in a population, as observed in Taiwan.

P0618. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy

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Early myoclonic encephalopathy (EME) with suppression burst (SB) is an early onset malignant epilepsy syndrome characterized by a typical EEG pattern, namely suppression-burst, in which higher-voltage bursts of slow waves mixed with multifocal spikes alternate with isoelectric suppression phases. The lack of consistent neuropathological features suggests that etiology may vary from case to case. EME has been reported in nonketotic hyperglycinemia (MIM 605899), propionic acidemia (MIM 606054) and some malformative disorders. However, in most cases, the underlying mechanism of these disorders remains unknown.

Here we report on genetic mapping of an autosomal recessive EME to chromosome 11p15.5 and identification of a missense mutation (Pro 206 Leu) in a gene encoding one of the two mitochondrial glutamate symporters (GC1, SLC25A22). The mutation co-segregated with the disease and altered a highly conserved amino-acid. Functional analyses showed that glutamate oxidation in patient cultured skin fibroblasts was strongly defective. Further studies in reconstituted proteoliposomes showed defective [14C]glutamate uniport and [14C]glutamate/glutamate exchange by mutant protein. Moreover, expression studies showed that, during human development, SLC25A22 is specifically expressed in the brain, within territories proposed to contribute to the genesis and control of myoclonic seizures.

These findings provide the first direct molecular link between glutamate mitochondrial metabolism and myoclonic epilepsy and suggest potential insights into the pathophysiological bases of severe neonatal epilepsies with suppression-burst pattern.

P0619. Rapid Throughput Sequence-Based Mutation Scanning of L1CAM: An Improved Method Based on Meta-PCR

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Mutations in the *L1CAM* gene are responsible for four related L1 disorders; X-linked hydrocephalus, MASA (Mental retardation, Aphasia, Shuffling gait, and Adducted thumbs) syndrome, X-linked complicated spastic paraparesis type I (SPG1) and X-linked Agenesis of the Corpus Callosum (ACC). The main clinical features of this spectrum are Corpus callosum hypoplasia, mental Retardation, Adducted thumbs, Spastic paraparesis and Hydrocephalus (CRASH). Since there is no biochemically assayed disease marker, molecular analysis of the *L1CAM* gene is the only means of confirming a clinical diagnosis.

The challenge for a comprehensive mutation scan in a large multi-exon gene such as *L1CAM* is to fulfil demand for rapid and accurate screening while reducing cost and turn-round time. Our previous mutation scanning technique involved the labour-intensive method of SSCP/heteroduplex analysis and sequencing of the 28 coding exons. Here, we report a cost-effective high-throughput direct sequence analysis strategy for *L1CAM*.

The whole coding region of *L1CAM* is amplified and sequenced in 8 separate fragments (6 Meta-PCR fragments and 2 long PCR reactions). Sequencing reactions are analysed on an ABI3730 automated sequencer and scanned for mutations using the trace subtraction software in the Staden Package.

Panels of 10 patients can now be analysed for all 8 fragments in approximately 8 weeks. To date, five previously unreported sequence alterations and one known pathogenic stop mutation have been identified from a total of 30 patients screened. One of these patients was a re-test with a previously negative SSCP/HA result, further confirming the increased sensitivity of this improved mutation scanning method.

P0620. Identification of a 3.0-kb major recombination hotspot in Sotos syndrome patients with a common 1.9-Mb microdeletion

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Sotos syndrome (SoS [MIM 117550]) is a congenital dysmorphic disorder characterized by overgrowth in childhood, distinctive craniofacial features and mental retardation. Haploinsufficiency of the *NSD1* gene due to either intragenic mutations or microdeletions is known to be the major cause. The common ~2.2-Mb microdeletion encompasses whole *NSD1* and neighboring genes and is flanked by low-copy-repeats (LCRs). Here we report the identification of a 3.0-kb major recombination hotspot within these LCRs, where we mapped deletion breakpoints in 78.7 % (37/47) of SoS patients with the common microdeletion. The deletion size was subsequently refined as 1.9 Mb. Sequencing of breakpoint fragments from all the 37 patients revealed junctions between a segment of the proximal LCR (PLCR-B) and its corresponding region of the distal LCR (DLCR-2B). PLCR-B and DLCR-2B are the only directly orientated regions, whereas the remaining PLCR and DLCR are in inverted orientation. PLCR with a size of 394.0 kb and DLCR of 429.8 kb showed overall high homology

(~98.5 %), with an increased sequence similarity (~99.4 %) within the 3.0-kb breakpoint cluster. Several recombination-associated motifs were identified in the hotspot and/or in its vicinity. Interestingly, an average of 10-fold increase of a translin motif over the normal distribution within the LCRs was recognized.

Furthermore, a heterozygous inversion of the interval between the LCRs was detected in all fathers with a child carrying a deletion in the paternally derived chromosome. The functional significance of these findings remains to be elucidated.

P0621. *MEFV* gene is a probable susceptibility gene for Behcet's disease

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Behcet's disease (BD) is a rare, chronic multisystem inflammatory disorder. Epidemiologic findings suggest that both genetic and environmental factors contribute to the development of BD.

The prevalence of BD is higher in the Middle Eastern and Mediterranean populations. Another chronic inflammatory disease Familial Mediterranean Fever (FMF), is also known to be highly prevalent in these populations. The prevalence of BD is higher in the FMF patient population than in populations known to be rich in BD. Both BD and FMF have some pathophysiological features in common and they result from inappropriate activation of neutrophils. Clinical manifestations of both diseases can mimic each other and coexistence of both diseases in the same patient has been reported.

Given that BD and FMF have similar pathophysiological, epidemiological and clinical features, we hypothesized that the gene responsible for FMF, *MEFV*, may also play a role in the pathogenesis of BD. Therefore, we screened common *MEFV* gene mutations (E148Q, M680I, M694V, and V726A) in 42 BD patients who had no symptoms or family history for FMF in addition to 66 healthy controls. Fifteen patients (36%) displayed *MEFV* mutations (9 M694V, 5 E148Q and one M680I) and mutation rates were significantly elevated compared to 66 (11%) healthy controls ($p=0.0034$).

The occurrence of frequent *MEFV* mutations in BD patients provides evidence that *MEFV* gene is involved in the pathogenesis of Behcet's disease.

	Patient Group	Control Group	P value
Mutation Frequency	15/27	7/59	0.0034
M694V	9(3*)/33	4/62	0.0367
E148Q	5/37	1/65	0.0321
M680I	1/41	2/64	0.9999

P0622. Mutation screening of the entire retinoblastoma gene (RB1) in 252 retinoblastoma cases

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Retinoblastoma is the most common cause of ocular malignancy in childhood occurring due to inactivation of both alleles of the tumour suppressor gene, RB1. Individuals with familial, bilateral or unilateral multifocal retinoblastoma are carriers of a germ-line mutation. Only a small proportion of isolated, unilateral retinoblastoma (IUR) patients carry a germ-line mutation, 1.7-17%, the majority having somatic mutations in tumour tissue only. In total, we have screened peripheral blood DNA from 144 patients with bilateral retinoblastoma and 108 patients with unilateral retinoblastoma. Our screening strategy has involved preliminary analysis by combined SSCP/heteroduplex analysis of all 27 exons of the retinoblastoma gene (RB1) followed by bidirectional sequencing of subsequent shifts. Recently, we have introduced RB1 dosage analysis to identify larger duplications and deletions of the gene. To date, we have identified mutations in 85% of patients with bilateral retinoblastoma and in 16% of patients with unilateral retinoblastoma. Additionally, we provide a service for mutation screening in DNA extracted from paraffin-embedded

retinoblastomas (for unilateral patients where germline screening has proved negative). We have identified both somatic mutations in 13 retinoblastomas of this type. We describe the detailed analysis of these cases. With the use of RB1 dosage analysis we hope to increase our mutation-detection sensitivity and provide an improved service to retinoblastoma patients.

P0623. L-2-hydroxyglutaric aciduria; Three Iranian cases and review of literature

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Background: L-2-Hydroxyglutaric aciduria is a rare and novel autosomal recessive inherited neurometabolic disorder. Since its first description by "Duran" in 1980 about 46 cases have been reported. Occurring mostly in childhood, it is characterized by slowly progressive neurological dysfunction with cerebellar ataxia, pyramidal sign, intellectual decline, seizure and extra pyramidal symptoms. MRI scanning is highly characteristic and screening for organic acid (L-2-Hydroxyglutaric acid) in urine, serum, and CSF is diagnostic.

Materials & Methods: We investigated three Iranian children aged (4, 14, 16 years) suspected of this rare disorder by urinary organic acid assay and MRI scanning. Symptoms were suggestive of one of the leukoencephalopathies accompanied with macrocephalia.

Results: Affected cases were evaluated because of mild to moderate psychomotor retardation and regression. Head circumferences were above 2 standard deviation. Urine levels of L-2-Hydroxyglutaric acid were strongly increased. MRI scanning of the brain showed hyper intense signals on T2 weighted images of the sub-cortical white matter and basal ganglia in all of them.

Conclusions: Because of its inheritance pattern (autosomal recessive), and the high rate of consanguineous marriages in Iran, the prevalence of this disorder may be high among mentally handicapped patients, especially those with macrocephaly. So we should consider this entity in differential diagnosis of mentally retarded patients with macrocephaly.

P0624. Analysis of some genes which may be involved in pathogenesis of sporadic amyotrophic lateral sclerosis in Russian population.

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The underlying causes of sporadic amyotrophic lateral sclerosis (SALS) remain unknown until now. Current evidence suggests that genetic factors triggering focal initiation and then spreading of motor neuron degeneration may be implicated in ALS pathogenesis. Various hypotheses have been suggested as potential contributors of disease such as oxidative damage and excitotoxicity. In this connection, such genes as hypoxia-inducible factor 1 (*HIF1A*) which plays an essential role in cellular and systemic homeostatic responses to hypoxia and may be involved in oxidative damage, glutamate transporter gene (*SLC1A2*) and ionotropic glutamate receptor genes (*GRIA1* and *GRIA2*) which are involved in excitotoxicity can play a causal role in SALS development. To investigate a role of these genes in SALS, we studied polymorphisms in these genes in 72 patients with SALS in Moscow and a related control population from Russia. The comparative analysis for IVS9-675C>A polymorphism of *HIF1A*, Gly603Ala polymorphism in the *SLC1A2* gene, SNP polymorphism (rs545098) of *GRIA1* gene and SNP polymorphism (rs9307959) of *GRIA2* gene has not revealed any significant distinction between the frequency of different alleles and genotypes for these gene polymorphisms in SALS patients and controls from Russia. We conclude that none of the investigated gene polymorphisms is associated with SALS and, probably, these genes are not involved in the development of SALS in the Russian population.

P0625. Analysis of His1096Gln mutation in Wilson disease patients from Russia.

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Wilson disease (WD) is an inherited autosomal recessive disorder caused by a defect in a copper transporting P-type ATPase (*ATP7B*). More than 100 mutations in the *ATP7B* gene have been identified in the disease. Most of these mutations are rare and were found in only a single family. But some mutations have been reported at higher frequencies: in Eastern Europe up to 38% of WD patients are homozygous for the substitution His1096Gln. To investigate a role of the His1096Gln mutation in the Russian population we have carried out screening for this mutation in 43 patients with "abdominal" WD and 120 patients with "neurological" WD. The frequency of the mutant allele 1096Gln is 25.6% in patients with "abdominal" WD and 41.6% in patients with "neurological" WD. The observed differences between the two groups are significant ($\chi^2 = 7.00$; $p=0.0082$). Moreover interesting differences have been revealed in the distribution of His1096Gln mutation genotypes between analyzed groups. Homozygous variants of His1096Gln mutation have not been identified in patients with "abdominal" WD, whereas in the patient group with "neurological" WD the frequency of homozygotes for this mutation was 18%. This difference in genotype distribution is statistically significant ($\chi^2=9.26$; $p=0.0098$). These data confirm that mutations of His1096Gln are the most frequent in patients with WD in Russia and are the main reason for the development of the disease.

P0626. Y Chromosome Microdeletion Analysis in Infertile Men

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30 % of cases that are diagnosed to have severe male infertility are due to genetic irregularities. Severe oligoasthenoteratozoospermia and azoospermia are accepted to be the most frequent symptoms in these cases. There are important genes that have roles in spermatogenesis on Yq11. It is thought a series of spermatogenetic disorders are related to microdeletions in this region. 12 azoospermic and 11 oligozoospermic cases were referred for genetic counselling to our department during 2001-2004. Two spermogram samples 2-3 weeks apart, hormone profiles, transrectal and scrotal USG examination when needed, and peripheral chromosomal analysis were performed. The following 15 sequence-tagged sites on the Y chromosome were analysed by PCR: AZFa (sY81, sY82, sY84), AZFb (sY127, sY142, sY164, RBM1), AZFc (sY145, sY152, sY153, CDY, BPY, DAZ1, DAZ2, DAZ3). Peripheral chromosome analysis revealed 47, XXY in only one case in the azoospermic group. Y chromosome microdeletions were detected in 4 out of 12 azoospermic cases (33%) and in 1 out of 11 oligospermic cases (9%).

Y chromosomal microdeletions are transmitted from a father to a son via ICSI and also the microdeletions may be expanded during such transmission. Genetic counseling for infertile couples contemplating ICSI is important if the male carries Y chromosomal microdeletions.

P0627. A 6.2-kb *TaqI* fragment of *CYP21* gene in congenital adrenal hyperplasia

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Approximately 75% of defective *CYP21* genes are attributable to the process of intergenic recombination of the DNA sequence from the highly homologous *CYP21P* pseudogene in congenital adrenal hyperplasia (CAH). Such a defective *CYP21* gene shows a 3.7-kb fragment produced by *TaqI* digestion from analysis of a PCR amplification product. Otherwise, *CYP21P*, mutations of IVS2 -12A/

C>G combined with 707-71delGAGACTAC, and the chimeric CYP21P/CYP21 gene show a 3.2-kb fragment. Therefore, the 3.7- and 3.2-kb fragments produced by *TaqI* digestion respectively are crucial markers of the CYP21 and CYP21P genes during the analysis of the C4-CYP21 repeat module. Herein, we present one CAH carrier with a CYP21 haplotype containing a 6.2-kb *TaqI* fragment, which was caused by a mutation of C to G at the *TaqI* site (TCGA) located downstream of the CYP21 gene as determined by analysis of a PCR amplification product and DNA sequencing.

P0628. ARNSD, GJB2 Mutations and the Δ (GJB6-D13S1830)

Deletion in Kurdish Population

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Introduction: Non-syndromic hearing impairment is the most common form of deafness, affecting approximately 1 in 1000 neonates. In approximately half of cases, the deafness is inherited, and of inherited congenital deafness, mutations in GJB2 make up the largest fractional contribution in many world populations. In this study, we analyzed 209 persons from 77 Kurdish families segregating severe-to-profound autosomal recessive non-syndromic deafness (ARNSD) to determine the frequency of GJB2 mutations in this population.

Mutation screening of GJB2 was performed by allele-specific PCR, with DHPLC analysis of all samples excluding 35delG homozygotes. Direct sequencing was completed on samples with abnormal elution profiles. The Δ (GJB6-D13S1830) mutation was identified by PCR-based amplification across the breakpoint region. We identified 6 mutations in exon 2 (35delG, R32H, delE120, R184P, R127H, and V153I). Based on these data, GJB2 mutations account for approximately 18.8% of severe-to-profound congenital deafness in the Kurdish population. We are going to continue our study on Non-GJB2 deafness family by (DFNB) Haplotype and linkage analysis to find out the other genes that may be defect in Kurdish population in the west of Iran.

P0629. Molecular studies in 11 Silver-Russell syndrome patients

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Introduction: Silver-Russell syndrome (SRS; Silver et al. 1953; Russell 1954) is characterised by intrauterine growth retardation, short stature in later life, typical craniofacial abnormalities including a relatively large, prominent forehead, a small triangular face, asymmetry of head and limbs, and other less constant abnormalities. The majority of cases of SRS are sporadic; occasionally a familial occurrence with autosomal dominant, autosomal recessive or X - linked inheritance is reported.

Objectives: To define the frequency of uniparental disomy (UPD) in the group of SRS patients.

Material and methods: Eleven patients with SRS syndrome and their parents were typed with short tandem repeat markers from chromosome 7. Chromosomal investigations were also performed in all patients.

Results: Maternal UPD was detected in one SRS patient (maternal uniparental heterodisomy), accounting for approximately 9 % of the tested SRS patients. In one patient the balanced chromosomal rearrangement 46,XY,der(5;8)(q31.3;q21.3)mat. was revealed.

Conclusion: The etiology of SRS syndrome has been heterogenous which makes diagnosis so far difficult. The responsible genes are still unidentified.

P0630. Molecular pathogenesis of metaphyseal dysplasia, Schmid type (SMCD)

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Schmid metaphyseal chondrodysplasia (MIM156500) is a dominantly

inherited cartilage disorder caused by mutations in the gene for the hypertrophic cartilage extracellular matrix structural protein, collagen X (*COL10A1*). Thirty heterozygous mutations have been described to date, almost equally divided into two mutation types, missense mutations and mutations that introduce premature termination signals. The *COL10A1* mutations are clustered in the 3' region of exon 3, which codes for the C terminal NC1 trimerization domain. The effect of *COL10A1* missense mutations have been examined by *in vitro* expression and assembly assays and cell transfection studies, which suggest that a common consequence is the disruption of collagen X trimerization and secretion, with intracellular degradation. We present data regarding the effect of *COL10A1* nonsense mutations in cartilage in two patients with SMCD, demonstrating that mutant mRNA is completely removed by nonsense mediated mRNA decay. We propose that, for both classes of mutations, functional haploinsufficiency of *COL10A1* in cartilage is the underlying basis of the clinical phenotype observed in SMCD.

P0631. Towards a genotype-phenotype correlation in individuals with Börjeson-Forssman-Lehmann Syndrome

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Börjeson-Forssman-Lehmann Syndrome (BFLS) is seen as an XLMRS with severe mental retardation in connection with progradient microcephaly, facial dysmorphology, gynecomastia, trunk obesity, hypogonadism, and without any development of an adequate language so far. BFLS is caused by mutations in the PHD protein family gene *PHF6*. The majority of published mutations are missense mutations or nonsense mutations, which lead to a truncated form of the protein. We had published the first triplet nucleotide deletion in *PHF6*, which caused only moderately mild symptoms of BFLS. This deletion maps to Exon 10 of *PHF6*. This part of the gene is not present in all *PHF6* transcripts, since at least two transcript isoforms of this gene exist. This deletion may be found only in isoform I.

Here we report on two other cases of BFLS: one sporadic case and another family with two affected sons, one healthy son, and their mother who as a carrier shows only very mild facial symptoms of BFLS. *PHF6* sequence analysis of the sporadic case revealed the recurrent R342X mutation. The mutation in the family with mild symptoms of BFLS is again a triplet nucleotide deletion in isoform I of *PHF6*. However, this mutation is different from the earlier published sequence variant.

It seems that severe cases of BFLS are due to single nucleotide changes in either isoform of *PHF6*, whereas mild form of BFLS may be diagnosed preferentially by a loss of nucleotide triplets in isoform I of *PHF6*.

P0632. Molecular confirmation of complete mole

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We used Fluorescent Quantitative PCR for confirmation of paternal uniparental diploidy at molar pregnancies. 15 different highly polymorphic STR markers (Ampfestr Identifier PCR Amplification Kit -Applied Biosystems, USA) together with amelogenin marker were co-amplified from DNAs isolated from product of conception tissue with karyotype 46, XX and mother's blood. Paternal uniparental diploidy was confirmed if all 15 STR polymorphisms of product of conception were monoallelic and more than one polymorphism could not be inherited from mother. Our method can reliably confirm paternal origin of 46, XX karyotype at product of conception with its significant clinical consequences. Partial and complete mole can be distinguished by ultrasound and histological appearance but cytogenetic and molecular genetic evaluation should be considered whenever there is a question of the diagnosis particularly to prevent malignancies.

P0633. Chaperone-procollagen interactions differ with mutation location in osteogenesis imperfecta

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Osteogenesis imperfecta (OI) or brittle bone disease, is caused by mutations in type I procollagen. The heterotrimeric procollagen

molecule consists of a central triple helical domain flanked by C and N-terminal propeptides and mutations in these regions results in OI. As both fibroblasts and osteoblasts secrete mutant collagen, the bone-specific pathophysiology of OI has not yet been delineated. However, in OI cases with mutations in the helical region of collagen, osteoblasts secrete a greater proportion of the mutant collagen forms than fibroblasts. As interactions with ER chaperones can direct the fate of proteins between secretion and degradation, we hypothesized that differential interactions of mutant procollagens with chaperones in osteoblasts and fibroblasts may be responsible for the 'permissiveness' of osteoblasts to mutant collagen survival.

Using confocal microscopy, collagen and chaperone-specific antibodies, we compared the intracellular localization of procollagens and chaperones in control versus OI fibroblasts. Normal procollagen and procollagen with a helical mutation displayed a distinct reticular pattern of immunofluorescence in the ER that overlapped with calnexin, but not with Hsp-47, PDI and BiP. In contrast, procollagens with C-propeptide mutations displayed a diffuse pattern of ER localization that co-localized with Hsp-47, PDI and BiP, but not with calnexin. These chaperone interactions are maintained in normal and OI osteoblasts. Our novel findings demonstrate a clear correlation between the type of mutation and both the subcellular localization pattern of procollagen and the nature of chaperone interactions in both fibroblasts and osteoblasts.

P0634. Leukemia inhibitory factor gene mutations contribute to embryo implantation failure in infertile women.

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The successful implantation requires a functionally normal embryo at the blastocyst stage and an adequately receptive endometrium. Their cross talk is complex, promoted mainly by cytokines produced and received by both, the endometrium, and the blastocyst. As it was shown, leukemia inhibitory factor (LIF) is one of the essential cytokines in this regulation. The defects in the embryo - endometrium communication may result in the implantation failure.

We designed a LIF gene mutation screening method that is based on the Temperature Gradient Gel Electrophoresis (TGGE). The population to screen consisted of 176 clinically characterized women with diagnosed infertility including a subgroup of 57 women with idiopathic primary infertility and history of infertility treatment and failure of in vitro fertilization (IVF) or intracytoplasmatic sperm injection (ICSI). The control population comprised of 75 healthy fertile subjects that conceived spontaneously and delivered successfully.

Six positive samples were detected by TGGE. The consequent DNA sequencing proved that all of them were potentially functional LIF gene mutations, the G→A transitions at the position 3400 of the LIF gene. This mutation causes the V64M exchange in the AB loop region of the LIF protein.

All six positive women were infertile. Four of them were diagnosed with primary and two of them with secondary infertility. No positive samples were identified in the control group.

The results suggest that LIF gene mutations can contribute to embryo implantation failure and consequent infertility and decreased pregnancy rates in ART.

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P0635. Osteocalcin gene HindIII polymorphism and bone mineral density in children with juvenile chronic arthritis in St.Petersburg.

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Osteopenia (OP), or low bone mineral density (BMD) for age, is a frequent complication in children with juvenile chronic arthritis (JCA). Osteocalcin is a biochemical marker that is often used in the assessment of bone turnover in OP patients. Previous studies have suggested an influence of osteocalcin gene *HindIII* alleles on bone metabolism. In the present study we investigated whether this polymorphism is related to BMD in JCA patients.

Seventy JCA children (51girls, 19 boys) were included in our study. The mean age of patients was 11.56 ± 4.17 years. Osteocalcin gene *HindIII* polymorphism and serum levels of intact osteocalcin, calcitonin, parathyroid hormone, Ca^{2+} , phosphate, common alkaline phosphatase was tested in all patients. OP was detected by dual-energy X-ray absorptiometry in lumbar spine (L1-L4).

Using the data of BMD we selected the children in two groups: with OP - 39 children (55.7%) and without OP - 31 children (44.3%). There weren't significant difference in genotypes frequency in those groups. But we investigated sex differences in genotypes distribution. There was significant difference in genotypes frequency between JCA girls with and without OP.

Genotypes	Boys with OP (n=13)	Boys without OP (n=6)	Girls with OP (n=26)	Girls without OP (n=25)	Children with OP (n=39)	Children without OP (n=31)
Presence of H (HH+Hh)	4 (30,8%)	4 (66,7%)	13 (50,0%)	7 (28,0%)	17 (43,6%)	11 (35,5%)
Absence of H (hh)	9 (69,2%)	2 (33,3%)	13 (50,0%)	18 (72,0%)	22 (56,4%)	20 (64,5%)
p	0,14		0,003		0,15	

P0636. Role of polymorphism in the CYP1A1, EPHX1 GSTM1, GSTT1 and GSTP1 genes in the development of chronic occupation bronchitis

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The aim of this study was to investigate the possible roles of metabolic gene polymorphisms in the development and progression of chronic occupational bronchitis

Polymorphisms in the genes encoding *CYP1A1*, *EPHX1*, *GSTM1*, *GSTT1* and *GSTP1* were investigated in Russian patients with chronic occupational bronchitis (N=95); chronic dust bronchitis (N=40) and chronic toxic bronchitis (N=50) and in ethnically matched healthy individuals (N=205) living in Ufa, Bashkortostan (Russian Federation) by PCR-RLFP.

The AG genotype of *CYP1A1* gene was associated with increased risk of chronic occupational bronchitis ($\chi^2=6.35$, $p=0.01$).

The frequency of the GG genotype of *GSTP1* was significantly higher in patients (8.4% compared to control 2.0%, $\chi^2=5.49$ $p=0.02$).

The combination of AA genotype of *CYP1A1* and GG genotype of *GSTP1* have associated with increased risk of occupation bronchitis ($\chi^2=4.66$ $p=0.03$; OR=4.21).

The distribution of the *EPHX1* and *GSTM1* genotypes did not significantly differ between patients and healthy subjects.

Our findings support that the polymorphisms of the *CYP1A1* and *GSTP1* genes, which code for enzymes with dramatically altered activities, probably play a substantial part in susceptibility to development and progression of chronic occupational bronchitis.

P0637. Mental retardation and autism associated with a 1.2 Mb Xq25 duplication encompassing the glutamate receptor gene GRIA3

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Chromosomal imbalances are a frequent cause of mental retardation (MR) but rearrangements smaller than 5 megabases (Mb) are frequently undetectable due to the limited resolution of routine chromosome analysis. Recently, genome-wide screening for submicroscopic deletions and duplications has become possible with array-based comparative genomic hybridisation (array-CGH). In the course of a systematic screening for submicroscopic chromosome imbalances in children with syndromic MR by array-CGH, we identified a small Xq25 microduplication in a boy with MR and autism.

The patient, a 15 year-old boy, was referred to our Department

because of developmental delay, speech delay, autistic disorder and sleep disturbances. He also presented facial dysmorphism and progressive scoliosis. High-resolution karyotype and FRAXA screening were normal. We first performed array-CGH using a DNA microarray with ~1 Mb resolution. Gain of chromosomal material was detected for two clones in Xq25. This result was confirmed using a tiling path BAC microarray covering the whole X chromosome and the duplication was found to span 1.2 Mb between BACs RP6-64P14 and RP13-158L7. The duplication was also detected in the phenotypically normal mother. However, she had a biased X chromosome inactivation (81%/19%), supporting the involvement of the duplication in the patient's phenotype.

Interestingly, the duplicated region encompasses the glutamate receptor subunit gene GRIA3. This gene has been proposed as a candidate gene for MR and bipolar disorder based on its disruption by a balanced translocation in a female patient. Our results provide additional evidence that abnormal GRIA3 expression may be involved in X-linked MR.

P0638. Y-position cysteine substitution in type I collagen (α (I) R888C) is associated with Type IV Osteogenesis Imperfecta

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The most common structural mutations in type I collagen causing osteogenesis imperfecta are substitutions for glycine residues in the uninterrupted Gly-X-Y triplets. X and Y position residues have been shown to be important for normal interchain interactions; it is not known whether substitutions at these residues cause or modify clinical conditions. We have delineated a Y-position substitution in a father and son with mild type IV OI. The 12 yr old proband and his father had sustained several fractures, and had osteopenia and moderate joint laxity. Both had an α 1(I) Arg888Cys substitution, which was confirmed in gDNA by MboI digestion. Proband type I collagen had less overmodification than expected from a glycine substitution at this position, supporting a minor effect on helix folding. There was also a faint, reducible α 1(I) dimer, which was demonstrated in about 10% of heterotrimers in media and cell layer of cultured fibroblasts by [³⁵S] cysteine labelling, compared to the theoretical maximum of 25%. In matrix deposited in culture, collagen containing mutant dimers and monomers efficiently formed mature crosslinks. Although differential scanning calorimetry revealed only local helix destabilization, in vitro processing of procollagens and western analysis of matrix deposition extracts suggest this mutation interferes with processing at propeptidase cleavage sites far removed from the mutation. In vivo, proband dermal fibril diameters have a wider range than in controls. These data suggest that X and Y position substitutions in type I collagen may have a significant clinical role as the cause or modifier of connective tissue disease.

P0639. The molecular-genetic analysis of congenital adrenal hyperplasia (21-hydroxylase deficiency)

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Congenital adrenal hyperplasia (CAH) is a group of recessively inherited diseases, in which cortisol secretion is impaired. More than 95% of all cases of CAH are due to deficiency of steroid 21-hydroxylase. The disease is caused by mutations in the CYP21 gene encoding the steroid 21-hydroxylase. More than 90% of these mutations result from intragenic recombinations between CYP21 gene and the closely linked CYP21P pseudogene. It was described that approximately 20%

of them are nonfunctional chimeric CYP21P/CYP21 genes due to unequal crossingover during meiosis.

We established a PCR based approach which permits differential amplification of the CYP21 and CYP21P genes, followed by direct probing for presence of known mutation sites in a secondary PCR analysis. The chimeric CYP21P/CYP21 gene, the deletion of the CYP21 gene and mutations 656A/C>G, P30L, G110del8, I172N, cluster E6 (I236N+V237E+M239K), V281L, Q318X, R356W, F306+1nt were detected in our patients. A total of 89 unrelated CAH patients from Czech Republic with 21-hydroxylase deficiency were examined. The most frequent mutation, chimeric CYP21P/CYP21 gene, was found on 68 mutant alleles (40.0%). Following frequent mutations were 656A/C>G (27.9%) and I172N (12.3%). After genotype-phenotype correlation it was found that the mutations P30L and V281L were characteristically identified in mild - late onset patients, the mutations 656A/C>G and I172N were usually seen in simple virilizing patients and remain mutations were presented in severe affected patients with salt wasting phenotype.

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P0640. Effects of C-propeptide Mutations in Type I Collagen on Extracellular Matrix Deposition and Fibrillogenesis

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Type I collagen C-propeptide mutations have been found in a small number of patients with Osteogenesis Imperfecta (OI). The mutations are not expected to be present in collagen fibrils in tissues, because the C-propeptide is cleaved before fibril assembly. Thus, their pathophysiological mechanism will differ from helical mutations. We identified 5 novel C-propeptide mutations by RT-PCR and sequencing of COL1A1 cDNA. Four involved substitutions at conserved residues: W1097C, D1233N (Type III OI), T1120I and P1266H (Type IV OI). The 5th proband (Type II OI) had a 6 nt deletion at the E51/I51 junction causing an in-frame insertion of all but 2 nt of I51 in cDNA. We compared proband fibroblasts with a lethal C-propeptide mutation (D1263Y; Pace, J Med Gen 2002), 3 mutations adjacent to the C-propeptide (G898S, G967C, G997S) and control cells. All mutant collagens showed backstreaking of α 1(I) steady-state collagen. Incorporation of pro- α 1 chains with point substitutions required 3-6X longer than control or G898S (~20 minutes). Pro- α 1 chains with an intronic insertion incorporated after 3 hours. Processing assays suggested delayed C-propeptide cleavage from secreted collagen containing C-propeptide or helical mutations. Overmodified collagens incorporated into fibroblast matrix in culture and formed mature cross-links. Skin and bone fibrils from two probands (T1120I, P1266H) were examined by EM. Proband dermal fibril diameters are ~10% larger than control. SEM revealed disorganized bone fibrils with variable diameters. These investigations provide insight into the pathophysiological mechanism by which mutations not expected to be incorporated into ECM structure alter matrix organization and weaken connective tissue.

P0641. Developing a successful screening strategy for X-Linked Retinitis Pigmentosa.

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Retinitis pigmentosa (RP) is the name for a genetically and phenotypically heterogeneous group of progressive retinal disorders with a combined incidence of approximately 1 in 3500.

There are autosomal dominant, autosomal recessive, X-linked recessive (XLRP) and sporadic forms of the disease and to date approximately 36 loci have been implicated.

XLRP is the most severe form of the disease with affected males showing symptoms before the age of 20 and with total blindness by the age of about 35. Mutations in the RPGR gene account for approximately 70% of XLRP cases, with a mutation hotspot in the highly repetitive region known as ORF15. A second gene, RP2, yields approximately

10% of XLRP mutations, with a mutation hotspot in exon 2. We have developed a high-throughput, semi-automated sequence detection strategy for detecting mutations in ORF15. To date over 130 cases have been screened and reported. To extend the service we are screening for mutations in RPGR exons 1-14 and RP2 exons 1-5. When this work is complete we aim to detect 80% of XLRP cases which will account for approximately 8% of all RP.

No effective approach to prevention, stabilization or reversal currently exists for the majority of RP cases and consequently pre-symptomatic testing is controversial. We ensure that all testing is offered within the context of genetic counselling and have demonstrated a high level of demand for this service in families who have previously found it difficult to obtain testing within an accredited laboratory environment.

P0642. Quantification of TSPY gene and probable identification of gonosomal aberrations in infertile men using capillary electrophoresis

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TSPY gene is a multicopy gene distributed on the Y chromosome in 6 clusters. In the normal population there are 20-40 copies. The TSPY gene is known for its role in testicular carcinomas and seminomas and probably also has role in spermatogenesis. We are investigating the relation of TSPY gene copies with azoospermia and oligospermia.

We used RQF PCR (Refined quantitative fluorescent PCR) to determine the copy number of TSPY genes. Patients with azoospermia and oligospermia were compared with normal fertile males. The AMEL gene on the X and Y chromosome was used as an internal quantitative control. In addition to the number of copies we were also able to identify chromosome aberrations and mosaics of sex chromosomes in our study.

We present our preliminary results in this poster; more detailed study is needed to reach conclusive results.

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P0643. Simultaneous detection of copy number changes and CpG methylation of all genes in the Chromosome 15 imprinted region with a novel method; Methylation-specific MLPA (MS-MLPA)

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Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS) are autosomal dominant disorders and the vast majority of the cases are caused by copy number changes in the chromosome 15 imprinted region or by uniparental disomy. The recently developed MLPA method has increased the possibilities for multiplex detection of copy number changes in a routine laboratory. Here we describe a novel robust method: the Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) which can detect changes in both CpG methylation as well as copy number of up to 40 chromosomal sequences in one simple reaction. In MS-MLPA, ligation of MLPA probe oligonucleotides is combined with digestion of the genomic DNA-probe hybrid complexes with methylation-sensitive endonucleases. Digestion of the genomic DNA-probe complex, rather than double stranded genomic DNA, allowed the use of DNA derived from formalin treated paraffin-embedded tissue samples. We successfully used MS-MLPA to detect copy number and methylation status of all genes in the chromosome 15 imprinted region in DNA samples of patients with Prader-Willi syndrome (PWS) or Angelman syndrome (AS).

P0644. Comprehensive mutation scanning in the ornithine transcarbamylase (OTC) gene using dye binding/ high resolution thermal denaturation

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Ornithine transcarbamylase (OTC, OMIM #311250) deficiency is an

X-linked (Xp21.1) inborn error of metabolism involving the urea cycle. The algorithm for differential diagnosis of urea cycle defects (UCDs) is complex. OTC deficiency is the most common UCD. Molecular analysis of OTC is useful as an initial reflex to clinical presentation of hyperammonemia. Dye-binding/high-resolution thermal denaturation (DB/HRTD) chemistry and the new high throughput LightScanner™ instrument were used to scan the OTC gene in 20 OTC deficient patients. Primers amplify the coding regions and a minimum of 12 bases of donor/acceptor splice site sequence. A single PCR product was used to amplify each of the 10 exons in a 96-well microtiter plate. A common amplification protocol is employed. OTC is X-linked, thus specimens from males were co-amplified with a known wild-type male control to force heteroduplex formation. Fragments ranged from 146-266 bp. The saturating dye LCGreen™ Plus was included in the PCR reaction. Post-PCR, the 96-well plate was transferred to the LightScanner for thermal denaturation. Fluorescent signal change was monitored during thermal denaturation generating a unique melting profile for each sample. Melting profiles were compared using automated analysis software. Samples with deviant profiles were recovered for DNA sequencing to characterize the causative nucleotide change. Nineteen mutations (13 reported, 5 novel) were identified. Known mutations: R23X, K46delA, IVS3+1G>A, R92X, R129H, N161S, A140P, N199S, IVS8+1G>T, C303G, R320X, V339L, and T343K. Novel mutations: R26K, L57Q, A217E, Q235H, E239G, and W265X. Rapid evaluation of OTC using DB/HRTD can speed diagnosis of OTC deficiency in affected patients.

P0645. Revised structure of human GLI2 gene reveals existence of the repressor domain and a novel mechanism for activator generation

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Mammalian Gli proteins are important transcription factors involved in Sonic hedgehog signal transduction pathway. Because of the association of Gli2 with mammalian development and with human disease, we have studied the structure and expression of human GLI2 gene. We show that human GLI2 contains a hitherto unknown repressor domain encoded by exons 3-6 which is well conserved among vertebrates. GLI2 has two alternative 5' noncoding exons, 1a and 1b. Additionally, in ovary and testis we detected two novel alternative splice forms of GLI2 generated by either skipping of exon 3, or exons 4 and 5. These two variants display different characteristics in the GLI-dependent transactivation assay. Our results suggest that in addition to proteolytic processing, alternative splicing may be another important regulatory mechanism for the synthesis of human GLI2 protein variants with or without repressor or activator properties.

P0646. Molecular genotyping of CYP21 gene in Slovak patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency.

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common inherited metabolic diseases. Enzyme 21-hydroxylase plays important role in the steroid biosynthesis of cortisol and aldosterone in adrenal cortex. Enzyme deficiency is caused by the defects in 21-hydroxylase gene (CYP21). About 90% of affected alleles are generated through non-homologous recombination with adjacent pseudogene CYP21P. We performed the first molecular-genetic analysis of 45 Slovak patients (88 unrelated chromosomes) to detect the mutations in CYP21 gene using PCR, allele-specific PCR, enzyme digestion and direct sequencing. Among 45 patients, 38 were diagnosed as the salt-wasting type, 4 as the simple virilizing and 3 as the nonclassical type. Genotyping analysis of 87 affected alleles revealed 12splice mutation to be the most frequent (43,7%) in our group of patients. Eighteen of 87 alleles (20,7%) carried large deletions and large gene conversions were found in 11,5% of affected alleles. Point mutations Ile173Asn, Leu308insT, Val282Leu, Gln319STOP and Arg357Trp were present

rarely, with the frequency 5.7%, 4.6%, 3.4%, 1.1% and 1.1% respectively. Two alleles carried combination of 2 mutations: 12splice+Val282Leu and Leu308insT+Gln319STOP. Genotype of 2 of 45 patients did not reflect the expected phenotype. PCR analysis was not able to detect any mutation in 5 affected alleles. Subsequent sequencing analysis of two of them revealed no sequence changes in comparison with reference CYP21 sequence. Further analysis is performed to detect potential mutation of the 3 remaining alleles.

P0647. Comprehensive alpha- and beta-thalassemia genotyping by means of reverse-hybridization teststrips

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Alpha- and beta-thalassemia (thal) are among the most common inherited diseases throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Mutations in the beta-globin gene, or in one or both of the two alpha-globin genes, are leading to structural abnormalities (e.g. sickle cell anemia) or to haemoglobin imbalance due to the reduced synthesis or complete absence of the respective globin chains. Unlike the prevalence of point mutations in beta-thal, the majority of alpha-thal alleles are derived from single or double gene deletions.

We have developed reverse-hybridization assays (StripAssays) for the rapid and comprehensive genotyping of alpha- and beta-thalassemia. The tests are 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. The tests are simple and convenient, and require very small amounts of samples, which is of particular importance for prenatal diagnosis. Although the spectrum of alpha- and beta-thal mutations is known to be highly population-specific, the broad range of variants covered by the StripAssays should make them globally useful diagnostic tools. (oberkanins@viennalab.co.at)

P0648. Attention-Deficit/Hyperactivity Disorder (ADHD) - Basal Genetic Aspects

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Attention-deficit/hyperactivity disorder is a clinical disorder characterized by an inattentiveness, impulsivity and hyperactivity. The occurrence of this disorder alters between 3 and 6% of the children population afflicted with boys predominating over girls at a ratio of 3:1 or more.

At present, the existence of more than thirty genes of the dopaminergic, noradrenergic, serotonergic and GABAergic systems is described which may take part in the development of the hyperkinetic disorder. In these genes, polymorphisms occur frequently that may affect function of gene products or modify gene expression.

We investigated the association between ADHD and the polymorphisms in the genes of the dopamine receptors (DRD2, DRD3, DRD4 and DRD5), dopamine transporter (DAT1) and dopamin-β-hydroxylase (DBH) involved in metabolism of dopamine and serotonin transporter (5-HTT). We examined the occurrence of the risk alleles in a group of 83 children with ADHD from Czech population at the age of 3 to 12 years and 92 controls. Also we assess the relationship between the presence of these alleles and the development and relevance of the hyperkinetic disorder and of comorbide psychiatric diseases.

Preliminary results suggest association between A1 allele of the TaqA1 polymorphism (DRD2) and ADHD in our study cohort. Homozygous individuals for this risk allele were more frequent in the patients than in the controls. In the other genes, risk alleles were presented at absolute majority in the group of ADHD children.

P0649. FBN1 mutation screening in infants and the importance of a family mutation database

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Mutations in the *FBN1* gene have been characterised at the molecular level in patients affected by Marfan syndrome and Marfan-related disorders. Starting with genomic DNA extracted from peripheral blood, we analysed the *FBN1* gene using PCR, SSCP and/or dHPLC analysis, and automatic sequencing of abnormal bands/peaks. Accurate molecular diagnosis is useful in long-term prognosis, genetic counselling and preventive healthcare.

We analysed a consecutive series of 451 patients, of which 18 were infants, and our results are comparable with those reported by other groups. Molecular diagnosis is especially useful for early diagnosis when the clinical symptoms are not yet evident.

A total of 210 relatives (of which 29 were infants) of 82 patients for whom a mutation had been identified, here or at another facility, were tested for the presence of that particular mutation. Prenatal and postnatal screening and tests in adults are very simple and straightforward, underlining the importance of knowing in advance the location of the putative mutation. The identification of a mutation allows for timely preventive management of mutation carriers and reassurance for unaffected family members.

Prenatal molecular diagnosis is carried out via chorionic villus biopsy or amniocentesis. After counselling, it is the patient's decision to consider termination when the mutation is clearly causative. A viable alternative is postnatal buccal swab diagnosis, which permits suitable treatment early in life if the mutation is present.

P0650. Reduced cajal body number in a patient with haploinsufficiency for COIL

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Here we report a one-way translocation t(11;17)(p15.15;q23.2)del(17)(q23.2)mat in a proband and mother with developmental delay and dysmorphic features, both haploinsufficient for COIL. COIL encodes for p80 coilin, the molecular marker of the Cajal Bodies (CBs), which are subnuclear domains that contain a wide variety of components, including factors involved in splicing, pre-rRNA processing, histone pre mRNA 3' maturation as well as transcription factors. The nuclear coiled body is located in the interchromatin space between the nucleolus and the nucleus. CBs have recently been implicated in the assembly and/or modification of the RNA-processing machinery.

The breakpoint on chromosome 17 was shown by Fluorescence In Situ Hybridisation (FISH) using bacterial artificial chromosomes (BACs) to include a deletion of ~1Mb, which includes COIL. The CBs of LBV transformed cell lines from the 2 translocation carriers were immunolabeled with anti-coilin antibodies and antibodies that recognise a CB U2 snrnp specific protein. Both haploinsufficient cell lines presented CBs in only ~2% of the cells, as opposed to the control cell line, which presented ~20%. A mouse knockout has shown that full-length coilin is essential for proper formation and/or maintenance of CBs and recruitment of snRNP and SMN complex proteins to CBs (Tucker et al. 2001). If CBs, key organelles involved in RNA processing in general, are reduced in haploinsufficient patients for COIL, and main molecules necessary for RNA processing are thus absent or reduced, this may lead to alterations in splicing and RNA transcription with potential phenotypic consequences.

P0651. MLC1: a novel protein in distal astroglial processes

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a progressive cerebral white matter disease in children. The disease is

histopathologically characterized by myelin splitting and intramyelinic vacuole formation. We have shown that the disease can be caused by mutations in the gene *MLC1*. Missense and splice site mutations, as well as various kinds of deletions have now been found in this gene. Furthermore, in some MLC patients without mutations in the intron-exon boudaries or exons the expression of the *MLC1* mRNA is reduced, as shown by quantitative RT-PCR suggesting mutations in regulatory elements outside the *MLC1* open reading frame. So far, there does not appear to be a correlation between the geno and phenotype. *MLC1* encodes a novel protein, with a putative transport function, which is mainly expressed in brain and leukocytes. *MLC1* is a plasma membrane protein, which contains an even number of transmembrane domains. *MLC1* is predominantly expressed in distal astrocytic processes in perivascular, subependymal and subpial regions. In gliotic tissue the expression of *MLC1* is upregulated. The localization suggests a role for *MLC1* in a transport process across the blood-brain and blood-CSF barrier. Elucidation of the function of *MLC1* will contribute to a better understanding of not only the pathophysiology of the disease, but also of the role of astrocytes in normal brain tissue. Our recent work is aimed at characterization of the possible transport function. Since there is evidence for a second gene involved in MLC, we have started a genome wide scan.

P0652. Epidemiology of Huntington's disease in Spain from the experience of symptomatic genetic testing

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Objectives: DNA molecular testing for Huntington's disease (HD) allows us to identify cases with atypical symptoms and/or absence of family history. The aim of this study is to provide information on the epidemiology of HD from the experience of nine years of direct testing.

Patients and methods: Determination of the CAG repeats of the *IT15* gene was performed in 317 patients by PCR analysis. In all cases, demographic, clinical and family history were carefully reviewed. The incidence of HD was calculated on the bases of the number of clinically symptomatic cases newly diagnosed by genetic testing per million inhabitants and per year.

Results: HD diagnosis (CAG repeat length ≥ 36) was confirmed in 52% of all symptomatic cases. Of them, 76 (45.8%) referred a positive family history and in 21 cases (12.7%) family history was negative. New mutation events were genetically proven in three families and highly suspected in another, estimating that the minimum new mutation rate for HD in our population is over 4% with a potential mutation rate of 8%. More than 16% of all HD cases had late onset (> 59 years). The incidence rate for the Autonomous Communities of Navarra and the Basque Country, based on the number of newly diagnosed cases by genetic testing, was 4.7 per million per year.

Conclusions: The incidence and mutation rates of the HD seem in our population is two to three times higher than previously reported for other European countries. Late onset of symptoms may be more frequent than previously estimated.

P0653. A novel *Sod1* mutation in a patient with Brachial Amyotrophic Diplegia

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Over 100 mutations have been described spreading through the entire *Sod1* coding region, but also in the intronic and regulatory regions. This gene, located on chromosome 21q22, is implicated in the autosomal dominant form with late onset of the Amyotrophic Lateral Sclerosis, ALS. Until now, all the *Sod1* mutations reported are associated to this pathological phenotype. In this study we describe for the first time a new *Sod1* mutation in a patient with Brachial Amyotrophic Diplegia (BAD). The patient was a 77-year-old man with a 5-month history of severe, bilateral arm weakness and wasting. Family history was unremarkable for neurological disorders. Neurological examination showed a peculiar posture of the "man-in-the-barrel". There was severe weakness and

atrophy of both upper limb muscles, especially of shoulder girdles (MRC<3/5). A wide screening was performed to exclude other causes of "man-in-the barrel syndrome". Molecular analysis of the survival motor neuron gene and X-linked spinobulbar muscular atrophy was negative. Genetic investigation of the five exons of the *Sod1* gene by DHPLC and direct sequencing of the PCR products, showed a variant profile of exon 4 caused by a heterozygous T→C substitution at position 1126 of the gene (Leu106Pro). This mutation was not found in more than 150 control subjects from southern Italy. In conclusion, *Sod1* mutations have been actually described in a small percentage of apparently sporadic cases of ALS and, our patient is the first case of sporadic lower motoneuron disease with a mutation in the *sod1* gene.

P0654. Mutations of the *PKD1* and *PKD2* genes in families with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. The disease is caused by mutations of *PKD1* (affecting roughly 85 % of ADPKD patients) and *PKD2* (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 237 different germline mutations have been reported. *PKD2* locus (MIM 173910) was localized to 4q13-23 and 61 different germline mutations were identified. Patients with *PKD2* mutation have milder clinical course (later onset of the disease and its complication) in comparison with *PKD1* patients.

>The direct detection of mutations in the non-duplicated region of the *PKD1* gene was performed in 78 nonrelated individuals. We detected ten mutations/polymorphisms in 32 families/individuals; 9 mutations/polymorphisms unique. We identified 2 nonsense mutations, 5 missense mutations/polymorphisms and 4 mutations/polymorphisms in splice site.

PKD2 mutation was performed in 121 nonrelated individuals. We detected twenty five mutations ; 10 mutations unique for Czech population. We identified the nonsense mutations in 9 patients, the frameshifting mutations in 13 patients and 3 missense mutations. Establishing 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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P0655. Different mechanisms preclude mutant *CLDN14* proteins to form tight junctions in vitro.

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Mutations in claudin 14 (*CLDN14*) cause non-syndromic DFNB29 deafness in humans. The analysis of a murine model indicated that this phenotype is associated with degeneration of hair cells, possibly due to cation overload. However, the mechanism linking these alterations to *CLDN14* mutations is unknown. To investigate this mechanism, we compared the ability of wild type and missense mutant *CLDN14* to form tight junctions. Ectopic expression in LM cells of wild type *CLDN14* protein induced the formation of tight junctions, while both the c.254T>A (p.V85D) mutant, previously identified in a Pakistani family, and the c.301 G>A (p.G101R) mutant, identified in this study through the screen of 183 Spanish and Greek patients affected with sporadic non-syndromic deafness, failed to form such junctions. However, the two mutant proteins differed in their ability to localize at the plasma membrane. We further identified hitherto undescribed exons of *CLDN14* that are utilized in alternative spliced transcripts. We demonstrated that different mutations of *CLDN14* impaired by different mechanisms the ability of the protein to form tight junctions. Our results indicate that the ability of *CLDN14* to be recruited to these junctions is crucial for the hearing process.

P0656. 769G→A Mutation in INHα1 and CGG - Repeats in FMR1 genes pilot testing in Ukraine

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Premature ovarian failure (POF) is characterized by loss of ovarian function before the age of 40 years. It occurs in 1% of all women, and in 0,1 % of women under age 30 years. The inhibin, is a potential candidate for POF due to its role in the negative feedback control of FSH, which has a pivotal role in the recruitment and development of ovarian follicles during folliculogenesis. The functional mutation in any of the inhibin genes would lead to a decrease in the amount of bioactive inhibin and as a result to increase in the concentrations of FSH and hence result in POF. As well it have been shown that fragile X permutations to occur more frequently in women with POF than in the general population. The frequency of 769G→A mutation in INHα1 among women population (n=61) was established as 5,6%. The CGG - repeats number in FMR1 gene analyses in group of 215 women (oocytes donors) revealed that the frequency of persons with high risk alleles (more 42 copies) is 2,3%. The results of our research can be the background for genetic testing of mutations in INHα1 and FMR1 genes among the women of reproductive age with the purpose of POF prognosis and/or prevention the birth of children with fragile X syndrome.

P0657. Informativity of FMF molecular diagnostics

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Familial Mediterranean Fever (FMF, MIM 249100) is recessively inherited and widely spread in Armenian population. FMF is an autoinflammatory disorder characterized by self-limited recurrent episodes of fever and serosal inflammation, caused by mutations in the MEFV gene, mapped on 16p13.3 locus. We have demonstrated the correlation between spectrum of MEFV mutations and clinical severity of the disease, including development of renal amyloidosis. In collaboration with Prof. Amselem and Dr. Cazeneuve (Hospital Henri Mondor, France) the complete screening of MEFV gene in patients with a clinical diagnosis of FMF (according to established criteria) identified a few patients without mutated alleles depending on the ethnic background. These results revealed the FMF-like syndromes in some cases without MEFV mutations.

Colchicine largely prevents the development of renal amyloidosis in FMF, but once the latter is established the effect of colchicines remains controversial. The homozygous genotype M694V/M694V (10 exon) is associated with a severe phenotype of FMF. We demonstrated that the genotype might predict response to colchicine given to children with renal amyloidosis. FMF patients homozygous for M694V mutation not only present a more severe phenotype but also show a limited response to colchicine at the nephrotic stage of renal amyloidosis. In contrast, FMF patients with other genotypes still have a good chance to enter remission of the nephritic syndrome and to maintain renal function.

In conclusion, our investigations on more than 5000 persons since 1997 confirm the informativity of screening of MEFV mutations for prevention, treatment and prognosis of the development of FMF complications.

P0658. Predominance of W24X and absence of 35delG mutations in the Baloochi and Sistani deaf population of Iran: a different population

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Hereditary hearing impairment is a heterogeneous disability showing different patterns of inheritance and involving a multitude of different genes. Mutations in the GJB2 gene, especially the 35delG mutation, have been established as a major cause of inherited and sporadic non-

syndromic deafness in various ethnic groups. Because population-specific differences are relatively common, in this study we sought to determine the prevalence and spectrum of GJB2 mutations in two isolated ethnic groups: the Baloochi and the Sistani populations of southeastern Iran. Consanguinity and assortative mating are very common in these populations. We analyzed one hundred Baloochi and Sistani families suffering from autosomal recessive non-syndromic hearing impairment. We performed mutation screening of GJB2 using an allele-specific PCR assay to detect the 35delG mutation. The negative or heterozygous cases for the 35delG mutation were screened by denaturing high performance liquid chromatography (DHPLC) and sequencing analysis. Surprisingly, we did not find the 35delG mutation, the most common GJB2 mutation in the white population and in other parts of Iran, in any of the Baloochi or Sistani patients. We identified GJB2 mutant alleles in 18 chromosomes (9%) including R127H, K122I, W24X, 167delT and M93I. Among them, W24X had the highest frequency (10 chromosomes). Based on these data, the hot-spot mutations in the GJB2 gene in the Baloochi and Sistani population with non-syndromic hearing loss may be different from other ethnic groups in Iran. In addition, these results further indicate the existence of an ethnic bias in the distribution of GJB2 mutations in western Asia.

P0659. Spectrum of GJB2 mutations in patients with autosomal recessive non-syndromic deafness in Yazd province of Iran

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Hearing loss is the most common sensory defect in humans, affecting 1 of 1000 neonates, with over half of these cases predicted to be hereditary in nature. Most hereditary hearing loss is inherited in a recessive fashion. Mutations in GJB2, encoding connexin26 subunit Beta 2, are major cause of inherited deafness in the most populations. We studied 95 probands from 95 families with autosomal recessive nonsyndromic hearing loss (ARNSHL) in Yazd Province, central Iran. Mutations Screening of GJB2 was performed by Amplification Refractory Mutation System (ARMS)-PCR to detect 35delG mutation. All samples excluding 35delG homozygotes were analyzed by DHPLC and sequencing. The frequency of GJB2-related deafness was found to be 7.4% in this population. We identified 5 mutations (35delG, 312del14, 314del14, R127H and 167delT) and three polymorphisms (V153I, V27I and E114G) in this study. Interestingly, 312del14, rather than 35delG, was the most common mutation found. Nine alleles (56.25% of GJB2 mutant alleles) carried the 312del14 mutation. In descending frequency, other common mutations were 35delG, 314del14, R127H and 167delT. The frequency of GJB2 mutant alleles was 9.5 % in this population. We conclude that: 1) 312del14 is common deafness causing GJB2 mutation in Yazd province; 2) this mutation may result from a founder effect; 3) The frequency of GJB2 mutations is very low in comparison to other parts of Iran. Further studies are needed to find other genes that have a causal role in ARNSHL in this population.

P0660. Comparison of different methods for the detection of PMP22 gene deletions and duplications

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By a dosage mechanism, overexpression of a duplicated PMP22 gene results in Charcot-Marie-Tooth disease type 1A (CMT1A) whereas underexpression because of PMP22 gene deletion causes hereditary neuropathy with liability to pressure palsies (HNPP). Several techniques can be used for molecular diagnosis of CMT1A and HNPP each having advantages and drawbacks. Multiplex ligation-dependent probe amplification (MLPA) is a method to detect gene dosage differences, by which not sample nucleic acids but probes added to the samples are amplified and quantified. We evaluated applicability of MLPA for the detection of the specific 1.5 Mb duplication/deletion present in CMT1A/ HNPP by comparing its results with those from interphase

fluorescence in situ hybridisation (FISH).

The sample included 70 patients referred with diagnoses of CMT1A or HNPP. Each patient was previously analysed with FISH using clone RP5-1005H15 (M. Rocchi) and with polymerase chain reaction (PCR) assay that detects a duplication specific recombination fragment. The MLPA was performed according to manufacturer's instructions.

A total of 9 duplications and 19 deletions were detected. There was 100% concordance between FISH and MLPA results. A single duplication was missed by the PCR assay which is in accordance with lower sensitivity of the PCR method.

The MLPA assay allows accurate detection of deletions/duplications of the gene PMP22. Therefore it should become an important method for molecular diagnosis of CMT1A/HNPP. The MLPA approach's primary advantages are the relative simplicity and speed although in some cases (i.e. mosaic conditions) FISH should remain the preferred diagnostic method.

P0661. Evidence for a founder effect of a deletion mutation associated with Crigler-Najjar Syndrome types I and II in Italian population

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Crigler-Najjar syndrome types I and II (CN1 and CN2; MIM# 218800 and 606785) are autosomal recessive conditions, characterized by non-hemolytic unconjugated hyperbilirubinaemia. CN1 is the most severe form, associated with the absence of hepatic bilirubin-uridined ipophosphoglucuronate glucuronosyltransferase (UGT1A1) activity. CN2 presents intermediate levels of hyperbilirubinaemia as a result of an incomplete deficiency of hepatic UGT1A1 activity. Here, we present the mutational analysis of *UGT1A1* gene in a cohort of 32 unrelated patients, originating from different Italian regions. The previously reported mutation c. 877T>A + 878_890del resulted segregating in five apparently unrelated Italian pedigrees. In addition a c. 878_890del was also identified in three patients. The c. 877T>A + 878_890del is likely to be prevalent in our CN1 syndrome patients (31%, 10/32 CN1 chromosomes), although we found heterozygous c.878_890del in three CN-2 subjects (in one case associated with a substitution 877T>A). This result suggests that the mutation c. 877T>A + 878_890del might be inherited from a common ancestor. We also hypothesized that the c.878_890del might be originated from the same mechanism. To test this hypothesis, we genotyped available members of the eight families for markers D2S2344, D2S206, D2S2348, D2S2205, D2S336, D2S2202 and D2S338. In addition, a set of single nucleotide polymorphism (rs4399719, rs4663971, rs4148328, rs6719561) were been tested in all patients.

Both mutations occurred on a shared haplotype which included D2S2348 and rs6719561 markers. The discovery of possible founder mutations has significant clinical applications, as a directed search for mutations is likely to account for the majority of carriers in the population.

P0662. A rapid molecular genetic test for the diagnosis of Klinefelter syndrome

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Klinefelter syndrome is the most common genetic cause of male infertility. Cytogenetic evaluation of metaphase chromosomes is in itself time-consuming, and the turnaround time is often very long. We have developed a reliable molecular genetic method to identify the presence of any extra X chromosomes, which can be completed in 3 working days.

DNA samples are isolated from blood and subjected to fluorescent 5-plex PCR for the amplification of the following markers: *amelogenin*, which is present in both sex chromosomes in a diallelic form; *X22*, a highly polymorphic pentanucleotide STR on the pseudoautosomal region of X and Y; *DXS6803* and *DXS6809*, two polymorphic X-specific tetranucleotide STRs; *SY134*, a Y-specific marker. The PCR products

are analyzed on an ABI 310 genetic analyzer. The conveniently designed product lengths and the two labeling dyes allow overlap-free marker identification. Automated marker designation and peak size labeling is carried out by a home-designed Genotyper template.

The presence of an extra X chromosome is diagnosed by either a supernumerary peak or an increased peak area based on criteria we developed. The quantitative feature of the test also allows the recognition of an identical extra X chromosome (error at meiosis II). Like with cytogenetic analysis, mosaic cases may not always test positive. In the past two years we identified 10 Klinefelter syndrome cases, all of which were confirmed by cytogenetic analysis.

While cytogenetics remains the standard method for the diagnosis of Klinefelter syndrome, our method offers rapid diagnosis on which hormonal and reproductive therapy can be based.

P0663. AP-1 and NF-kappaB activation and pro-inflammatory gene expression in foetus tracheal CF cells.

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AIMS: Cystic fibrosis is a common autosomal recessive disorder due to a mutation in the *cftr* gene, coding for a chloride channel. The *CFTR* channel dysfunction is associated with chronic airway obstruction, infection and inflammation. Since inflammation is present before and even in the absence of pathogens, the elucidation of the inflammatory molecular mechanism could lead to a strategy to prevent fibrosis in CF patients.

METHODS: We analyzed tracheal epithelial cells isolated from cystic fibrosis (CFT-2) and normal (NT-1) human fetuses for transcription factor activation. We also performed a micro-array gene analysis (U133 Plus arrays, Affymetrix).

RESULTS: We demonstrated by mobility shift that CF cells display high NF-kappaB and AP-1 activities compared to the control cell line. Increased NF-kappaB activity was linked to a high IKK activity and a short IKB-a half life. The gene expression analysis confirmed that many pro-inflammatory genes are expressed at higher levels in CFT-2 cells than in NT-1 cells. These gene code for cytokines, chemokines and inflammation linked enzymes.

CONCLUSION: Our data then confirm that pro-inflammatory mechanisms are activated in CF tracheal cells independently of any infection or stress.

P0664. Quantitative analysis of *TSPY* gene in Turner syndrome patients

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The *TSPY* gene exists in multiple copies in several clusters, with assumed splicing variability. The role of the *TSPY* gene is not fully understood yet, but it is supposed to be involved in directing spermatogonia into meiosis. Abnormal expression of *TSPY* was reported in gonadoblastomas. Our project analysed the percentage of *TSPY* loci in different tissues of Turner syndrome patients. DNA from 135 peripheral blood samples, from seven ovarian and tumour samples embedded in paraffin (4 resp. 3), and two RNA specimens from dysgenetic *TSPY*-positive patients were collected. QF PCR of *TSPY* loci and positive controls were used for quantitation. Eleven out of 135 patients were positive for Y sequences with extreme range of mosaicism or number of *TSPY* gene copies. Four ovarian and tumour samples out of 7 were *TSPY* positive, with a range from 0.01 % to 6 %. Tests of two cDNA samples were weakly positive in comparison with negative control. The *TSPY* gene is a significant candidate for GBY, indicating a possible role of *TSPY* in the multistage development of gonadoblastoma and dysgerminoma in the dysgenetic gonads of TS patients. Diagnostic analysis of Y sequences in TS patients should include the *TSPY* locus. For better understanding of gonadoblastoma tumour genesis more detailed specific study of the *TSPY* gene will be required. Supporting grant: Grant agency MH CR, NR/7821-3.

P0665. Investigation of Interferon-Gamma Receptor-1**Polymorphism in Iranian patients affected with Pulmonary Tuberculosis**

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Background: Tuberculosis is one of the most common infectious diseases in the world. Environmental and host genetic factors involve in susceptibility to infection disease such as tuberculosis. In recent years, genetically approach has been developed and it has been reported that some genes and their variants involve in this susceptibility. One of the interesting genes for investigator is IFN- γ R1. Our studies were to determine susceptibility to tuberculosis with polymorphism of IFN- γ R1 gene.

Material and Method: Study was prospective case-control. 54 patients with smear positive tuberculosis have been chosen randomly. All of patients were chosen based on having Iranian nationality, age above 15 yrs and confirmed smear positive pulmonary TB and all of healthy controls were matched in nationality and age with samples. DNA was extracted from whole blood and PCR-RFLP technique used to determine polymorphism at 395 codon of IFN- γ R1 gene. Data were analyzed with SPSS version 11.

Results: Mean age of patients and control were 55 ± 20 and 53 ± 13.5 years respectively. Demographic characteristic had no difference within two groups. (P-Value >0.05) one patient in case group had heterozygote mutation at IFN- γ R1 gene. In control group there were no mutations.

Conclusion: Genetically susceptibility to TB is not in 395 colon of IFN- γ R1 in Iranian TB sample and polymorphism of this loci has occur in 2% of TB patients and 0.96% of total study population.

P0666. Methylene tetrahydrofolate reductase C677T mutation is more frequently observed in recurrent abortuses than Factor V Leiden and Prothrombin 20210 G/A mutations

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Miscarriage is common in 25% of conceptions without an identifiable reason. Recurrent pregnancy losses have been investigated widely since two or more successive losses affect about 5% of women of reproductive age. Anatomical, chromosomal, endocrinological or immunological problems consist only a small percentage of pregnancy losses. In addition to these factors, thrombophilic predisposition may be one of the underlying causes of fetal losses.

In the present study we have determined the prevalence of Factor V Leiden (FVL), Methylene tetrahydrofolate reductase (MTHFR) C677T and Prothrombin (Prt) 20210 G/A mutations by PCR-RFLP method in DNA samples from 66 patients (aged between 22 and 43 years with two or more pregnancy losses) who applied to our routine diagnostic laboratory. Our preliminary results strongly indicate that heterozygous MTHFR C677T mutation occurred more often in patients with fetal losses (30 patients - 45.45%) while no difference in the prevalence of Factor V Leiden (6.06%) and Prothrombine 20210 G/A (6.06%) mutations were detected with respect to the results normal Turkish population. The high prevalence of mutated MTHFR genotypes in fetal losses may emphasize the potential protective role of periconceptional folic acid supplementation.

P0667. Some aspects of spermatogenesis and male infertility in Ukraine

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In about 50% of couples infertility is entirely due to male factors. In addition to chromosomal anomalies, microdeletions in the azoospermic factor region (AZF) in the long arm of the Y-chromosome have been detected in men with azoospermia or severe oligospermia. We have screened 160 men with azoospermia and oligospermia - patients of “ISIDA-IVF” clinic involved in ICSI (intracytoplasmic sperm injection)

program. The samples were analysed for Y-chromosome microdeletions by multiplex PCR. For this investigation 13 primer pairs were used that are homologous to previously identified and mapped sequence tagged sites (STS). The STS primers tested on each sample were sY84, sY85 (AZFa); sY117; sY124, sY134, USP9Y (AZFb); sY141, sY153; sY240, sY146, sY254 (DAZ), sY255 (DAZ), sY158 (AZFc). SRY was used as an internal control of PCR reactions. The PCR products were analyzed on a 1.8% agarose gel. In seven of the 270 infertile men (2.6%) we found one deletion in AZFb and 6 deletions in AZFc (gene DAZ) regions - thus the majority are concentrated in the AZFc region. We conclude that: a) genes damage in the AZFb region results in blocks of last stage spermatogenesis; b) deletions in the AZFc region are critical for early stage spermatogenesis. The genetic consultation and preimplantation sex analysis were recommended for the patients where Y-chromosome deletions were found.

P0668. A novel mutation in the sodium-channel gene SCN1A in a patient with severe myoclonic epilepsy (SMEI)

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Severe myoclonic in infancy (SMEI) is a distinct epileptic syndrome with the following principal features: normal development before onset; seizures beginning during first year of life in the form of generalized or unilateral febrile clonic seizures; secondary appearance of myoclonic seizures; and often partial seizures. SMEI is intractable and is associated with ataxia and mental decline. Recently, mutations were found in the gene (SCN1A) encoding voltage-gated sodium channel alpha-subunit type I in patients with SMEI. Those mutations were heterozygous and *de novo*. Here we describe the clinical features and genetic study of a patient with SMEI from southern Italy. The patient is an 11 year old boy who was born in Sicily. Onset of seizures was between 2 and 5 years of age with unilateral clonic seizures or generalized clonic or tonic-clonic seizures. The convulsions were often induced by fever, atypical absences were observed and the seizures were resistant to therapy.

Mutational analysis was performed on all coding exons and splice sites of SCN1A. We detected a novel heterozygous point mutation C3866T that resulted in a Thr1289Ile aminoacid substitution. This mutation was not observed in any of the 50 control individuals. The mutant residues Thr1289Ile is located in S4 transmenbrane segments of the sodium channel alpha-subunit. The S4 segments of voltage-gated channels contain multiple positively charged aminoacids that are known to have a role in channel gating.

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P0669. Molecular analysis of the Hexa gene in Italian patients with Tay Sachs disease: Detection of thirteen novel mutant alleles

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Tay Sachs disease (TSD; MIM # 272800) is an autosomal recessive inherited lysosomal disorder, characterized by hexosaminidase A (Hex A) deficiency, that results from mutations in the HEXA gene which encodes the alpha subunit of the beta-N-acetylhexosaminidase A. We report the molecular characterization of the HEXA gene in 31 Italian patients, twenty-two with the classical infantile TSD form and nine patients with the late onset form (B1 Variant). Of the 29 different alleles identified, thirteen were due to fourteen novel mutation including two mutations being *in-cis* on a new complex allele, missense/null mutations, deletions, a single base insertion and splicing mutations.

The missense mutations p.W203G, p.A246W, p.Q374R resulted in drastic changes in highly conserved residues among the human, mouse and rat α -subunits of Hex A. The 3D- structural analysis confirmed that all three mutations alter the substrate binding site.

The clinical presentation of the patients studied at the diagnosis was

similar to what have been already reported in literature: hypotonia, seizures, delayed milestones, cherry-red spots and startle reactions. A clear genotype-phenotype correlation was observed in the B1 Variant group: all patients carried the known c.533G/A (p.R178H) mutation, confirming its milder nature. It is interesting to note the contrast between the allelic homogeneity of the B1 Variant group (c.533G/A allele frequency: 75%) and the infantile TSD, in which almost all the mutations are private and have been detected in a single or few families.

P0670. Genetic analysis of three Serbian families with GEFS+

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Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome transmitted as an autosomal dominant trait with incomplete penetrance. GEFS+ is characterized by heterogeneous phenotypes with most individuals having benign childhood seizures disorders ranging from classical febrile seizures to febrile seizures plus where febrile seizures continue past 6 years of age or afebrile convulsions occur. GEFS+ is associated with mutations in SCN1A, SCN1B (genes encoding respectively the alpha 1 and beta 1 voltage-gated sodium channel subunits) and GABRG2 (gamma subunit of the GABA_A receptor).

Here we aimed to study the role of SCN1A, SCN1B and GABRG2 in 3 Serbian families with GEFS+. The 26 exons of SCN1A, 5 exons of SCN1B and 9 exons of GABRG2 were individually amplified using primers based on intronic sequences. The purified PCR products were directly sequenced and analyzed with an automatic sequencer. We analyzed the proband of each family and we identified polymorphisms but no mutations. These data suggest that SCN1A, SCN1B and GABRG2 genes are not involved in the aetiopathogenesis of GEFS+ in these families. In the meantime we recruited others member of the largest family and we are performing a genomewide genetic-linkage analysis to identify a novel GEFS+ locus in this family.

Supported by MIUR-FIRB "Inherited channelopathies: identification of molecular markers and functional development of advanced diagnostic systems, and identification of pharmacological targets"

P0671. Mutational analysis of EPM2A and NHLRC1 genes in three patients suffering from Lafora progressive myoclonus epilepsy

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Lafora's progressive myoclonus epilepsy (LD) is an autosomal-recessive disorder caused by mutations in the *EPM2A* gene. An additional causative gene for Lafora's disease was recently identified, termed *NHLRC1* or *EPM2B*. It is a single-exon gene, encoding a putative E3 ubiquitin ligase (malin). In this study, we screened *NHLRC1* in three patients with LD who did not carry mutations in *EPM2A*. Probands are three Sicilian patients who received a diagnosis of LD after a comprehensive clinical and laboratory investigation. In two of them, age at onset of LD was 14 years, in the remaining patient LD started at the age of 11 years. Sequence analysis of *EPM2A* did not reveal any pathogenic variant in all three patients. Then, we proceeded to sequence the *NHLRC1* gene. We sequenced the whole *NHLRC1* exon, including non-coding sequences. In one patient we found a homozygous nonsense mutation (G199T, giving rise to a stop codon at aminoacid residue 67). The second patient was a compound heterozygote for two mutations (C205G causing the P69A substitution, and G838A resulting in the E280K change). The last patient carried the heterozygous E280K mutation and a known polymorphism (C332T leading to the P111L substitution). All the mutations except the latter polymorphism were not found in 100 control chromosomes. Regarding the last proband, the P111L change represents a very common polymorphism and we found the pathogenic E280K variation in a heterozygous state. *Supported by Ministero della Sanità - "Genetica dell'Epilessia"*

P0672. DJ-1 Gene in late-onset recessive Parkinson's disease

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Mutations in *DJ-1* were described as a novel cause of early-onset parkinsonism. We analysed the *DJ-1* gene in 23 subjects with late onset (after the age of 45 years) Parkinson's disease (PD) screened from 18 families originating from Southern Italy with autosomal recessive PD (LORPD). These patients were evaluated previously for the presence of parkin mutations (*PARK2*) by a combination of gene dosage and sequencing and were found to be negative. The 18 families with LORPD were selected according to the following criteria: reported parkinsonism in two or more siblings; a mode of inheritance compatible with autosomal recessive transmission (affected siblings without affected parents); an age at onset of 45 years or older in all the affected siblings. The entire *DJ-1* open reading frame was amplified from genomic DNA, and PCR products were directly sequenced by means of forward primers. We did not find any pathogenic mutation in the *DJ-1* gene in any patients with LORPD. We identified at codon 98 (exon 5) a G/A heterozygous substitution in four patients; this change was confirmed by *Msp* I restriction analysis, and was found in 10 out of 700 control chromosomes. Moreover we identified an 18 bp insertion/deletion variant in the promoter region of *DJ-1* (g.168_185del) in six patients; this variant was also found in 3 out of 600 control chromosomes. Our results indicate that mutations in *DJ-1* are not a common cause of LORPD. Further studies using quantitative PCR are needed to exclude heterozygous exon rearrangements in subjects with LORPD.

P0673. Hematopoietic chimerism analysis after allogeneic peripheral stem cell transplantation in pediatric cases by using STR polymorphisms

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Allogenic hematopoietic stem cell transplantation is carried out in patients with malignant and non-malignant hematologic diseases. Recently, peripheral blood has increasingly been used as a stem cell source in children. In this study, PCR analysis of polymorphic short tandem repeats (STR) was applied to 20 sex matched donor and recipient pediatric cases, with beta-thalassemia (n=10), immunodeficiency (n=2), adrenoleukodystrophy (n=1), juvenile myelomonocytic leukemia (JMML) (n=1), aplastic anemia (n=1), Fanconi aplastic anemia (n=1), osteopetrosis (n=1), acute myeloid leukemia (n=1), acute lymphoblastic leukemia (n=1), and non-hodgkin lymphoma (n=1). Analyses were performed at different time points after transplantation, with a total of 55 samples studied. Fifteen tetranucleotide repeat loci (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA) and Amelogenin were amplified by AmpFISTR®Identifier™PCR kit, and analyzed on an ABI PRISM®310 Genetic Analyzer. Calculations were based on relative areas of donor and recipient peaks. Patients are evaluated according to the cytogenetic, molecular cytogenetic and molecular results and clinical findings. Of the 20 patients, complete chimerism in 9 cases (45%), partial chimerism in 9 cases (%45) and no chimerism in 2 cases (%10) was observed in an average follow up time of 11.3 months. Interestingly, in one JMML patient with complete chimerism, donor cell derived JMML developed. We conclude that after peripheral stem cell transplantation, STR based analysis of chimerism is an important tool in post-transplant hematopoietic chimerism quantification, leading to early detection of graft failure, relapse, donor cell derived disease as well as minimal residual disease.

P0674. SOX9 gene dosage effect in 20 XX-SRY negative sex reversal patients

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The early developing gonads are sensitive to gene dosage, and threshold levels of expression exist to control entry into the male or

female pathway. The sex determining genes SRY and SOX9 are required to initiate and maintain the testicular development. SOX9 is the critical downstream target of SRY and is up-regulated specifically in the XY gonad shortly after the onset of SRY transcription. SOX9 haploinsufficiency is associated with campomelic dysplasia and XY sex reversal (75%). A single case has been reported in a XX sex reversal patient due to a duplication of 17q23-24, containing SOX9. To evaluate the SOX9 gene dosage in testis determination we performed Q-PCR analysis using Taqman MGB probe and RNase P as internal control in 9 XX males and 11 XX true hermaphrodites (TH). All patients were SRY negative. The SOX9 gene dose was established comparing the $\Delta C_t = (SOX9 C_t - RNase P C_t)$, $\Delta \Delta C_t = (\text{patient } \Delta C_t - \text{control } \Delta C_t)$ and the $2^{-\Delta \Delta C_t}$ formula. Preliminary results showed that 3 XX males and 4 TH had an heterozygous duplication of SOX9, while 1 XX males and 1 TH showed an homozygous duplication. This is the first report in which gene dosage effect of SOX 9 was analyzed in XX - SRY negative sex reversal patients suggesting that SOX9 duplications could induce testis determination in the XX primordium.

P0675. Scanning for Association of Mitochondrial Haplogroups BM, J, K and M with Multiple Sclerosis: Interrelation between Haplogroup J and MS in Persian Patients

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Background. Multiple Sclerosis (MS) is an immunological inflammatory disease of the Central Nervous System, chronically observed in young adults. On the basis of earlier studies, potential relatedness between MS and mitochondrial DNA (mtDNA) mutations was postulated.

Materials and Methods. 246 individuals were examined using PCR-RFLP, including 70 MS patients for mitochondrial haplogroups BM, J, K and M and 176, 149 and 70 normal controls for haplogroups BM and M, J and K respectively.

Results. Our analysis revealed a high proportion of haplogroup BM in MS patients (~26%) compared to normal controls (~13%) at $p=0.027$. There was also a slightly significant correlation found between MS patients and normal controls for haplogroup J (20.00% for patients vs 9.39% for controls at $p=0.049$), while haplogroups M and K didn't appear relevant in MS occurrence (2.85% and 2.27% respectively at $p=1.000$ for haplogroup M and 12.85% and 7.14% respectively at $p=0.399$ for haplogroup K).

P0676. Study on Mediterranean Mutation as the Major Mutations in G6PD Polymorphic Variant Identified in the Three Provinces in South, East and South East of Iran

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G6PD enzyme catalyses the conversion of Glucose-6-phosphate to 6-phosphogluconate, with production of NADPH and ribose -5-phosphate in the red blood cells in the pentose phosphate pathway. Its gene consists of 13 exons. G6PD deficiency is the most common human metabolic inborn error. Acute hemolytic anemia and Jaundice are the main clinical symptoms, triggered by infection or ingestion of Fava beans or oxidative drugs. Three predominant variants of G6PD called Mediterranean, Chatham and Cosenza have been evident in several countries including Iran. Molecular identification of the most prevalent mutations in G6PD gene was carried out in 121 males and females with G6PD deficiency from Khorasan, Sistan-Baluchestan and Hormozgan provinces in south, east and south east of Iran. DNA was extracted from blood samples and analyzed for known G6PD mutation by RFLP. Adapting this method revealed that Mediterranean mutation at nt 563(C->T) is predominant in these areas (72.7%). This finding,

which is the first investigation in east and south east of Iran, indicates a higher prevalence of this mutation in these provinces compared to the Coastal provinces of Caspian Sea (75.4%) by which we reported earlier. We are now conducting further studies for identifying other mutations. The distribution of this G6PD variant is more similar to that found in an Italian population in comparison with other Middle Eastern countries. Although the origin of Iranian population is rather uncertain, the closer similarity of the mutation spectrum to Italian rather than middle eastern populations may indicate that these populations have a common ancestral origin.

P0677. Searching for mutations of Cx32 and MPZ genes in families with Charcot-Marie-Tooth neuropathy from Tadzhikistan

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The purpose of the study was to search for mutations in the coding sequences of *Cx32* (Xq13.1) and *MPZ* (1q21.3-23) genes, which cause Charcot-Marie-Tooth (CMT) neuropathy, in a group of patients with CMT from Tadzhikistan and their relatives. Tadzhikistan is situated in South-East Asia at Pamir mountains; and the population of this region is ethnically isolated. We studied 35 patients from 23 families, in general 114 individuals. Analysis of *Cx32* revealed nucleotide changes in five families. Sequencing revealed C to T substitution in the second part of coding exon which resulted in Arg142Trp change (loss of Hpall site). In four families the mutation was detected in hemizygote state in sick men, while sick women as well as asymptomatic female carriers were heterozygous. A silent mutation (C to T substitution in the third position of Asp169) was identified in the same fragment in two healthy sibs in a big family. In the fifth family, single-nucleotide change G>A in the third part of coding exon was revealed, leading to change Arg219His (loss of Acil and Cac8I sites). In general, 21% of CMT-families in Tadzhikistan have mutations in *Cx32*. In *MPZ*, sequencing of exon 5 in 5 families revealed a heterozygous silent change G>A in Gly200. In summary, we describe 2 different missense (1 in 4 families, 1 in one family) and 1 silent mutation in exon 2 of *Cx32*, and 1 silent mutation in exon 5 of *MPZ* in patients from Tadzhikistan.

P0678. The Effect of ACE I/I Genotype on OSAS

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Obstructive Sleep Apnoea Syndrome (OSAS) is a disease characterized by repeated obstruction of upper respiratory tract during sleep. There is a quite limited number of studies on role of genetic factors in the etiology of OSAS. The aim of our study is to find out if ACE gene polymorphism and plasma ACE activity create susceptibility to OSAS in Turkish population. Ninety-seven patients, in total, were included in this study. The Epworth scores, ages, body mass indexes, neck circumferences, apnea-hypopnea indexes (AHI), average apnea durations, and minimum SpO_2 of the patients were recorded. Insertion (I)/Deletion (D) polymorphism of the ACE gene and plasma ACE activity levels were determined. When distribution of the I/D ACE polymorphism in our patients was compared to that of healthy controls from a previous study in Turkish population, it was observed that risk of OSAS development increased 2.8 times in patients who had I/I genotype of ACE gene. The average ACE activity in the patient group with I/I genotype was observed to be significantly lower than that of the group with D/D genotype ($p=0.019$). ACE activity decreased significantly in patients with severe OSAS, compared to those with mild OSAS ($p=0.017$). No statistically significant difference was observed upon comparison of I/I, I/D, D/D genotypes and minimum SpO_2 , neck circumference, Epworth score, average apnea duration, and AHI ($p>0.005$). As a consequence, these findings support that ACE I/D polymorphisms may have a role in the pathogenesis of the OSAS.

P0679. DFNB1-causing allele variants in the Iranian Turk population

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Mutations in the GJB2 gene at the DFNB1 locus have been established as the major cause of autosomal recessive non-syndromic hearing loss in many world populations. In addition to GJB2 mutations, the deletion Δ (GJB6-D13S1830) involving GJB6 gene (also localizes to DFNB1 interval) has been detected in many patients heterozygous for one GJB2 deafness-causing mutation. The aim of this project is to study the prevalence of GJB2 mutations in the deaf Turk population living in the East and West Azerbaijan provinces of Iran. Mutations screening began by amplification refractory mutation screening PCR to detect the 35delG mutation. We then analyzed all samples excluding 35delG homozygotes by DHPLC and direct sequencing for other GJB2 mutations. We screened 276 chromosomes (138 probands) for GJB2 mutations. Seventy five chromosomes (27%) carried GJB2 mutations including 35delG, delE120,-3170G>A, W24X, 363delC, E129K, Q80L, Y155X. Among them, 35delG had the highest frequency and Q80L and 363delC were novel mutations which have not been reported in any other populations. Thirty five patients had biallelic GJB2 mutations (25.3%) and five probands had monoallelic GJB2 mutation. The Δ (GJB6-D13S1830) was not found in any heterozygous patients. Polymorphisms found were V153I and V27I. Based on our results, other genes are likely to play a major role in causing nonsyndromic deafness in this population.

P0680. MLPA analysis of the dystrophin gene in DMD/BMD patients from Serbia and Montenegro

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We have designed an Multiplex Ligation-dependent Probe Amplification (MLPA) assay to simultaneously screen all 79 DMD exons for deletions and duplications in Duchenne and Becker muscular dystrophy (DMD/BMD) patients. We validated the assay by screening 133 patients from Serbia and Montenegro previously screened using multiplex PCR. MLPA-screening confirmed the presence of all previously detected deletions. In addition we detected seven new deletions, 10 duplications, one point mutation, and we were able to precisely determine the breakpoints of all rearrangements (21 were unknown from the previous study). Overall, unrelated patients from Serbia and Montenegro contained 64% deletions and 7% duplications, figures not significantly different from other studies. In two brothers we identified a complex rearrangement involving two duplications and a triplication. To facilitate MLPA-based screening in laboratories lacking specific equipment, we designed the assay such that it can also be performed using agarose gel analysis and ethidium bromide staining. The MLPA-assay as described provides a simple and cheap method for deletion and duplication screening in DMD/BMD patients. The assay outperforms the Chamberlain and Beggs multiplex-PCR test, and should be considered as the method of choice for a first DNA analysis of DMD/BMD.

P0681. A novel missense mutation (N258S) in the KCNQ2 gene in a Turkish family afflicted with BFNC

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cal School, İstanbul, Turkey, ⁴Institute of Human Genetics, University of Bonn, İstanbul, Germany.

Benign familial neonatal convulsions (BFNC) is a rare monogenic subtype of idiopathic generalized epilepsy exhibiting an autosomal dominant mode of inheritance. The disease is caused by mutations in the two homologous genes KCNQ2 and KCNQ3 that encode the subunits of the voltage-gated potassium channel. The heteromeric assembly of these subunits form the M-current which is the primary regulator of neuronal excitability. KCNQ2 is located on chromosome 20q13.3 and composed of at least 18 exons, comprising over 50kb of genomic DNA. This study reports the analysis of the KCNQ2 gene in a Turkish family with three BFNC patients in three successive generations. SSCP studies of the complete KCNQ2 gene indicated a migration shift in exon 4 of two patients. DNA sequence analysis of the region revealed an A>G transition that resulted in a novel substitution of serine instead of asparagine at position 258, which is between the S5 domain and the pore of the potassium channel. The absence of the mutation in healthy members of the family and in a control group of 60 German and 77 Turkish individuals excludes the likelihood of it being a polymorphism and indicates that N258S substitution is the pathogenic mutation leading to epileptic seizures in this family.

P0682. Novel homozygous mutation associated with a common variant in familial lecithin:cholesterol acyltransferase (LCAT)deficiency

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LCAT deficiency is an autosomal recessive disorder of lipoprotein metabolism, resulting from loss of function of lecithin:cholesterol acyltransferase, a key enzyme in extracellular cholesterol metabolism and cholesterol reverse transport. The human LCAT gene has been mapped to chromosome 16q22. The gene consists of 6 exons and encodes a protein of 416 amino acids. In the present study, we report the phenotypic and genetic characterization of one Italian patient and his relatives with classical clinical manifestations of familial LCAT deficiency. The diagnosis was confirmed by biochemical data: the patient's α -LCAT activity and CER were totally absent, while plasma levels of HDL-C, ApoA-I, ApoA-II and ApoB were severely reduced. Molecular analysis of the LCAT gene was performed. Sequence analysis revealed a novel mutation, in the proband and his brother. This mutation is a nucleotide T-to-G transversion (g.4896, c.1187 T>G), converting codon 372 for leucine, a hydrophobic amino acid, into a codon for arginine, a basic amino acid (p.L372R). This novel mutation is near the catalytic site H377. This substitution of Leu372 with Arginine, a positively charged amino acid with a different steric size, may affect the typical α/β hydrolase fold configuration of the catalytic triad and cause the loss of LCAT activity.

Interestingly, the two brothers were also homozygous for a common variant in exon 5 (p.S208T), never detected in homozygous status. In conclusion, our findings suggest that homozygous p.S208T variant could act as modifier allele, amplifying the effect of the p.L372R mutation and increasing the risk of cardiovascular events.

P0683. Two point mutations in the vWF gene leading to Normandy Type von Willebrand Disease

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von Willebrand disease (vWD) is a common autosomally inherited bleeding disorder characterized by a qualitative and quantitative deficiency of vWF protein in the plasma. Three primary vWD types are type 1, type 2 and type 3. Normandy Type results from mutations in the vWF gene region coding FVIII binding site. The protein vWF consists of eleven domains. Each domain in subunits plays a role in interaction with, proteins and in the multimerization of vWF itself. For example, the FVIII binding site is found within the mature vWF subunit and encoded by exons 18 to 23 of the gene. In this study, three patients, who are two

sisters and a brother, clinically diagnosed as vWD and suspected to be Normandy Type were tested for mutations in exons 18-21 and 24 of the vWF gene. The sequencing results in all three patients revealed a C→T transition at n. position 2446 in homozygous condition. This study confirmed Normandy Type of vWD in this family by DNA analysis. In addition, DNA samples of 3 hemophilia A patients who did not have inversions or point mutations in their F8 gene as a result of complete DNA sequencing, were tested for mutations in the same exons of the vWF gene. Sequencing results indicated that, one patient had a T→C transition at n. position 2362 in homozygous condition. This study showed that hemophilia A was a misdiagnosis for this patient.

P0684. Molecular pathology of acute recurrent and chronic pancreatitis in children. The *CFTR*, *SPINK1*, *PRSS1* and *SERPINA1* genes defects analysis.

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Mutations in four genes: *PRSS1*, *SPINK1*, *CFTR* and *SERPINA1* are thought to be a cause or a factor predisposing to pancreatitis. The aim of this study was to evaluate the impact of these defects into molecular pathology of chronic (CP) and acute recurrent pancreatitis (ARP). 92 children with CP or ARP and 55 family members were investigated. In 31 patients (33.7%, P=0.001) we identified mutations in at least one out of four examined genes. Mutations in the *SPINK1* and the *PRSS1* gene were found most frequently (8.7% vs 6.5% of total alleles). *PRSS1* mutations were identified mainly in CP patients, while the N34S *SPINK1* mutation was recognised with comparable frequency in CP and ARP patients. The frequency of identified mutations in the *CFTR* alleles was similar to the control group (4.9% vs 5%, P=1.000). Frequency of mutations E264V and E342K in the *SERPINA1* gene was lower than in the control group (P<0.001). Analysis of disease course in patients with identified mutations in the examined genes and their families showed, that presence of molecular defect (especially, the N34S *SPINK1* mutation) is not always associated with disease phenotype. The *PRSS1* defects seem to be strongly causative for CP or ARP whereas defects in *SPINK1* and *CFTR* are suggested to be only disease-modifiers. The *SERPINA1* variants are not associated with CP or ARP.

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P0685. Molecular Analysis of MeCP2 Gene in Patients with Rett Syndrome

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Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder caused by mutations in *MeCP2*, encoding methyl-CpG-binding protein 2. RS affects almost exclusively females with an incidence of one in 10 000-15 000 and more than 99.5% of cases are sporadic. Mutations in the *MeCP2* gene were identified in approximately 35-80% of sporadic RS cases. Because the *MeCP2* gene is subject to X chromosome inactivation (XCI) in females, the severity of the disease varies with the type of mutation and the pattern of XCI.

In this study, the genetic basis of Rett syndrome was investigated in samples from 24 sporadic classical RS patients (2 male and 22 females). Screening of the *MeCP2* gene in these patients using RFLP, SSCP and subsequent sequencing analyses revealed eleven point mutations, three novel deletions and a silent polymorphism (S293S). All patients were heterozygous for the mutations except one patient that was heterozygous for mutations P152R and R106W. They were found to be *de novo* mutations since the family members tested negative for them. Total skewed pattern of XCI was determined in nine patients and six of them were carrying *MeCP2* gene mutations. Intact mutant mRNA was found to be present in patients with novel deletions by RT-PCR analyses. The findings were in accordance with the previously published data. Patients with skewed XCI and TRD domain mutations

were more severely affected compared to patients having random XCI and missense mutations.

The present study will help our understanding of the molecular basis of RS in Turkish population.

P0686. Caring for PKU families in the National Research Center: an Egyptian Experience

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Many countries have reported extensive data about PKU, describing the scope and problems of screening to the mutational patterns. However, no large-scale data has been reported from Middle East and Africa. This presentation focuses on PKU in Egypt, as a model developing country. A team working in the National Research center cares for about 100 PKU families within this context and trying to reach an optimum economic perspective.

The clinical group offers genetic counseling, guided diet management, growth and neurological assessment and IQ evaluations. For each patient, phenylalanine level estimation was used for biochemical diagnosis and monitoring.

A molecular study targeting six mutations (IVS10, R261Q, R252W, Y277D, E221G and V245V) was initiated using PCR and restriction analysis. The results revealed the presence of IVS10 (17%), V232V (17%) and R261Q (7%). VNTR analysis was analyzed among PKU and normal individuals. The 7, 8 and 9 repeat units were the most common among PKU cases whereas 9, 3 and 7 were prevalent among normals.

Small-scale manufacturing was initiated to locally produce low phenylalanine food and milk products with promising success.

A support group was founded by Dr Laila Effat and interested parents to offer help to PKU families and to raise the awareness and knowledge of PKU in the community, particularly the issue of preventing mental retardation with early diagnosis. This multifaceted approach lays background for future implementation of PKU screening program in the country and sets an example for tackling this problem in a third world country.

P0687. Detection of mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia

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Congenital neutropenia and cyclic neutropenia are disorders of neutrophil production predisposing patients to recurrent bacterial infections. Recently, mutations have been detected in the neutrophil elastase (*ELA2*) gene in cyclic neutropenia and severe congenital diseases.

Peripheral blood was obtained from 21 patients with cyclic and congenital neutropenia. Total RNA was isolated from fresh-polymorpho-prep separated cells using standard RNA techniques. RNA was analysed by PCR amplification of reverse transcribed RNA using a total of ten specific primers. Mutation analysis was performed by direct sequencing. We amplified five exons of the *ELA2* gene separately and sequenced each exon. We found most mutations in exons 3 and 5 and a lower number of mutations in exons 2 and 4; no mutation was found in exon 1.

In conclusion, mutations in the neutrophil elastase gene were found in all the patients we studied. However in congenital neutropenia the mutations were more severe.

P0688. Molecular Analysis of the Williams Syndrome Critical Region through Fluorescence *in situ* hybridization and quantitative Real-Time PCR.

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Williams Syndrome (WS) is a contiguous gene deletion disorder caused by haploinsufficiency of several genes in 7q11.23, including the elastin locus (ELN). Clinically the WS-phenotype is characterized by elastin arteriopathy, supravalvular aortic stenosis, connective tissue abnormalities, dysmorphic face, growth and mental retardation, visuo-

spatial and cognition impaired and behavioural problems. About 95% of the patients with the classical phenotype present a deletion of WS Critical Region (WSCRD). Most patients had the WSCRD of 1.5 Mb. Nevertheless, recent studies have demonstrated a deletion of ~ 850 Kb. The aim of the present study was to identify through fluorescence *in situ* hybridization (FISH) and quantitative real-time PCR (qPCR) the WSCRD in a sample of 16 Mexican patients with clinical diagnosis of WS. FISH analysis was performed with a LSI probe that included the ELN, LIMK-1 and D7S613 genes. qPCR was done with FKBP6, CLDN3, and GTF2I probes and RnaseP probe as internal control. The gene dosage were established comparing the ΔCT =(gene CT-RnaseP CT), $\Delta\Delta CT$ =(patient ΔCT -control ΔCT) and $2^{-\Delta\Delta CT}$ formula. Fourteen patients harboured the WSCRD, one patient had no deletion and the other one showed apparently an atypical pattern of deletion. In conclusion, we propose that the gene dosage analysis mediated qPCR allows a more accurately determination of the WSCRD size.

P0689. Molecular profile of hypothyroid myopathy

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Background: an overt myopathy during hypothyroidism may occur. The mechanisms whereby decreased circulating levels of thyroid hormones may interfere with muscular function are still uncertain. Although the molecular actions of thyroid hormones have been studied thoroughly, their pleiotropic effects are mediated by complex changes in expression of unknown number of target genes.

Objective: the purpose of the study is to study muscular gene expression in hypothyroid myopathy in order to elucidate the pathogenesis of such a disease.

Materials and Methods: three groups of patients with hypothyroid myopathy were subdivided in relation of TSH levels: patients with TSH lower than 100 mU/l; patients with TSH about 100 mU/l; patients with TSH upper than 100 mU/l. Muscle specimens from age- and sex-matched normal subjects undergoing orthopedic surgery will be used as controls. Microarray experiments were performed using amplified RNA isolated in muscle specimens. A GeneChip microarrays panel of cDNA human Gene Array containing approximately 40000 genes from human genome (MWG-Affymetrix) was used.

Results: we found a correlation between TSH levels and the number of genes down-regulated. Patients with TSH levels upper than 100 mU/l had at least 1500 down-regulated genes. These were involved in energy metabolism, mRNA maturation, signal transduction cellular trafficking. **Discussion:** Microarray analysis is an effective tool for identifying genes differently involved in the response of skeletal muscle to hypothyroidism

P0690. Might there be a link between mannose binding lectin polymorphisms and vitiligo?

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Mannose binding lectin (MBL) is a calcium dependent lectin that causes predisposition to infections and autoimmune diseases. In this study we aimed to examine the presence of any association between *MBL* gene variants and vitiligo. Codon 54 (allele B) and codon 57 (allele C) polymorphisms in exon 1 of the *MBL* gene, were investigated in samples from 50 healthy controls and 40 patients diagnosed as having vitiligo. PCR-RFLP was used in order to investigate the polymorphisms in the *MBL* gene. The B allele frequency was significantly higher in the patient group (20%) compared to the control group (3%). The AB genotype was found 35% and 6% of the patient and healthy control groups, respectively. These results suggest that the codon 54 polymorphism in the *MBL* gene may play a role in susceptibility to vitiligo.

P0691. Mutation detection in the Neutrophil Elastase gene, *ELA2*, in an Iranian case affected with Kostmann syndrome

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Originally described Kostmann in 1956, the disease is a rare autosomal recessive disorder of neutrophil number. The absolute neutrophil count is characteristically less than 500/mm³. Severe persistent neutropenia results in an increased susceptibility to frequent bacteria infections. Neutropenia occurs in 1-2 cases per million population.

Heterozygous mutations in the gene, *ELA2*, located on 19p13.3, encoding neutrophil elastase, are the commonest cause of severe congenital neutropenia, a leukemia predisposing condition. Mutation of the *ELA2* gene prevents the maturation of neutrophils before they can enter circulation.

Here we describe a patient with Kostmann syndrome who demonstrates somatic mosaicism in the bone marrow of multiple different *ELA2* sequences representing acquired mutations of constitutionally wild type alleles. The proband is the first and only offspring of a second cousin couple. Initial infection occurred shortly after birth, and fever began on the second day. A typical pattern of recurrent fevers, Urinary Tract Infections, Irritability, localized site(s) of infection, Pneumonia, Boils, Cutaneous abscess and Omphalitis have been observed. Increased bone marrow monocytes (9%), and eosinophils (3%) and decreased neutrophils (2%) also have been observed. The patient is suffering from severe anemia, growth retardation and malnutrition. The patient, from an ethnically isolated population where recessive inheritance might be expected, has Kostmann Syndrome in the absence of a family history of neutropenia. Sequencing of PCR-amplified genomic DNA from peripheral blood that has been done in the University of Washington School of Medicine by Dr. Marshall Horwitz revealed two novel, apparently heterozygous *ELA2* mutations, V69L and V72L.

P0692. Screening for Cys 282Tyr and His 63Asp mutations of *HFE* gene in Kazakhs and Uygurs populations of the Central Asia

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Hereditary hemochromatosis (HH) is an autosomal recessive genetic disorder of iron metabolism. Two major missense mutations in *HFE* gene responsible for HH have been described: Cys282Tyr, accounting for 80%-90% of HH chromosomes, and His63Asp, which is associated with a milder HH form comprising from 40% to 70% of HH chromosomes. The availability of molecular genotyping for accurate HH diagnosis has led to increased interest in population screening to promote early diagnosis and therapy of this disease. For evaluation of carrier frequencies we have screened for Cys282Tyr and His63Asp mutations in *HFE* gene in populations of Kazakhs (N=260) and Uygurs (N=116) in Central Asia. We have detected both of the mutations in the populations studied, suggesting that hereditary hemochromatosis is likely to be present in Asian populations. The frequency of Cys282Tyr mutation carriage was 2.4% in Kazakhs and 1.7% in Uygurs. The frequency of Cys282Tyr mutation is lower than in European populations (8.65%) and in Turkic speaking populations of the Volga-Ural region (3.95%). We detected His63Asp mutation in heterozygous state in 16.0% of Kazakhs and in 16.4% of Uygurs, which is a lower frequency than in Europe (24.75%) and the Volga-Ural region (21.70%). These data confirm the decreasing frequencies of these two mutations from north to south and from west to east. These results led us to recommend performing of molecular genetic testing in groups at risk.

P0693. Molecular and cytogenetic analysis of Leukemic patients for 7 common translocations

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We have analysed 304 leukemic patients by multiplex reverse

transcription-polymerase chain reaction(RT-PCR) method for rapid diagnosis and screening of patients with 7 chromosomal translocations including: t(1;19)(q23;p13), t(12;21)(p13;q22), inv(16)(p13;q22), t(15;17)(q21;q22), t(9;22)(q34;q11), t(8;21)(q22;q22), t(4;11)(q21;q23) . Many of these translocations are related to acute and chronic, myeloid and lymphoid leukemias. Multiplex RT-PCR detects hybrid mRNAs transcribed from the fusion genes produced by translocations. Parallel cytogenetic analysis was performed on 120 of 304 patients too. Leukemic cell samples from patients admitted to Karimnejad-Najmabadi clinic were subjected to RNA isolation and cDNA synthesis were performed by random priming method on total RNA. To achieve maximal sensitivity a nested PCR protocol was used and a housekeeping gene was considered as internal positive control . Bone marrow culture were done and prepared cells were harvested with conventional methods and slides were prepared by GTG technique and studied.

We have studied 304 patients with the following indications and results:

From 216CML patients 109(50.5%) was positive for t(9;22)(q34;q11), from 65 AML patients 3(4.6%) was positive for inv(16)(p13;q22) and 3(4.6%) was positive for t(8;21)(q22;q22) and 15(23%) was positive for t(15;17)(q21;q22), from 23 ALL patients 1(4.3%) was positive for t(9;22)(q34;q11) , 1(4.3%) was positive for t(4;11)(q21;q23) and 2(8.7%) was positive for t(12;21)(p13;q22).

From 304 patients 120 cases were analysed cytogenetically and correlation between these results will be discussed.

RT-PCR is rapid and sensitive method for screening of translocations and is also the best tool for monitoring of minimal residual disease but other numerical and structural aberrations can be detected by cytogenetic analysis.

P0694. A Pilot detection study of alpha(1) antitrypsin deficiency in a targeted population in Iran

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Alpha-1-antitrypsin deficiency (A1AD) is a genetic disorder which is transmitted in an autosomal recessive pattern. Alpha-1-antitrypsin deficiency affects mainly the lungs and the liver leading, in the latter case, to neonatal cholestasis, chronic hepatitis or cirrhosis. Worldwide, approximately 1 in 2,500 individuals has alpha-1 antitrypsin deficiency. While this disorder is found in all ethnic groups, it occurs most frequently in whites of European ancestry. Estimates of the prevalence of A1AD (especially the PiZZ phenotype) have varied considerably, depending on the ethnicity of the population used to derive the estimate. Whether the A1AD is a very rare or is a widely under-diagnosed disease in Iranian population still is unknown. A pilot study was carried out on 75 unrelated children clinically characterized by idiopathic liver dysfunctions suspected to A1AD. Biochemical tests including enzyme assay were performed to identify the deficient cases. Molecular diagnosis tests of the Z and S mutation of A1AD were performed in all of the samples using RFLP-PCR method. The result indicates the rare incidence of Z and S mutation in Iran.

P0695. Werner Syndrome and mutations of the WRN and LMNA genes in a european population.

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Werner Syndrome (WS) is a rare hereditary disorder of premature aging. The clinical spectrum includes small stature, acute voice and a characteristic facies, plus the early onset of cataracts, diabetes, skin changes, grey hair, and other signs. WS can be difficult to diagnose due to the variability of the patients' presentation and the rarity of the disorder. Transmission is usually autosomal recessive.

The gene for WS, *WRN*, encodes a member of the RecQ helicase family involved in DNA replication. Biallelic mutations of *WRN* are responsible for most patients, though in up to 40 %, no mutation can be demonstrated. Recently, heterozygous missense mutations in the nuclear envelop proteins lamin A and C, both encoded by the *LMNA* gene, have been observed in WS patients with severe symptoms

and cardiac involvement, as well as in the more severe Hutchinson-Guilford progeria syndrome. Blood samples from fourteen WS cases were sent to us for molecular analysis. All 35 exons of the *WRN* gene and their splice junctions were amplified and sequenced. Four cases presented biallelic disruptive mutations. In three cases, only one mutated allele of *WRN* was identified. As dominant alleles of *WRN* have not been described, it is likely that a second mutation for each of these cases escaped detection, possibly due to large deletions, or to point mutations in the introns affecting splicing. The cases with no or only one mutated *WRN* allele are being analysed further for mutations in *LMNA*.

P0696. Mutations in the hemochromatosis gene (HFE) and multiple sclerosis

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In the present study we have investigated whether HFE gene polymorphism may play a role in the disease behavior of Croatian and Slovenian MS patients and their potential genetic susceptibility to MS. We genotyped 314 MS patients and 400 healthy controls for the C282Y and H63D mutations by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) analysis. Our results showed no significance differences in the distribution of the two mutations between MS patients and controls suggesting that HFE polymorphisms do not influence the susceptibility to MS. However, we observed that MS patients carrying the mutant C282Y allele exhibited earlier onset of disease symptom relative to others genotypes. Also, our data indicate that a gradient of risk of iron overload associated with different HFE genotypes may contribute to the progression of MS, but it warrants further study in a larger series of MS patients.

P0697. Mutations in AHI1 gene of Joubert syndrome patients.

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Joubert syndrome (JS) is a recessively inherited disorder characterised by hypotonia at birth and developmental delay, followed by truncal ataxia and cognitive impairment, and suggestive facial features. Abnormalities on axial MRI are the neuroimaging hallmarks of JS, and include cerebellar vermis hypoplasia and abnormalities at the pontomesencephalic junction that lead to a characteristic "molar tooth" appearance. JS is clinically heterogeneous with some patients presenting with various combinations of breathing abnormalities in the neonatal period, oculomotor apraxia, retinal dystrophy, retinal coloboma, ptosis, hexadactyly, and nephronophthisis or cystic dysplastic kidneys. JS is also genetically heterogeneous, with two known loci, on 9q34 (JBTS1) and 11p11-q12 (CORS2), representing only a fraction of cases. We have identified a third locus in 6q23 (JBTS3) from the study of two large consanguineous families of Turkish and Swiss origin. LOD score calculation for the two JBTS3 families, including the consanguinity loops, gave a maximum value of 4.1 and 2.3 at $\theta = 0$, respectively, indicating linkage between the disease and the D6S1620-D6S1699 haplotype spanning a 13.1 cM interval. Recently, Ferland et al. (2004) and Dixon-Salazar et al. (2004) found mutations in the gene AHI1 in 6 families with Joubert syndrome linked to 6q23. We are sequencing AHI1 in JS patients from 10 families. We have found so far a homozygous Serine to Stop mutation (S783X) in exon 17 and a truncating mutation 630-633delGAAA in exon 7 in the linked Turkish and Swiss families, respectively. Sequence analyses of the other 8 families is in progress.

P0698. Identification of mutations in the galactose-1-phosphate uridyltransferase (GALT) gene in 15 Iranian patients with galactosemia

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Classical galactosemia caused by deficiency of galactose-1-phosphate uridylyltransferase (GALT) is a severe autosomal recessive disorder. We report here molecular analysis of 15 unrelated Iranian galactosemia cases confirmed to have galactosaemia.

Molecular testing is done in two steps: 1. The six most common mutations including Q188R, K285N, S135L, L195P, X380R and Q169K are first tested using PCR-RFLP method. 2) In order to determine the unknown mutations the entire coding region of GALT was subjected to the sequencing.

The most common molecular defect observed in the Iranian population was Q188R (replacement of glutamine-188 by arginine) (60%). One homozygote for S135L mutation, one heterozygote for K285N mutation, three rare mutation and the presence of a deletion in the coding region of the GALT gene were detected.

P0699. Functional analysis of a human *atp12* mutation in yeast *S. cerevisiae*.

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Complex V (ATP synthase) of the respiratory chain couples the proton gradient, generated by the respiratory chain, to ATP synthesis. The *ATP12* gene product is one of the proteins required for assembly of complex V. Here, we report on the functional effect of a W94R mutation, present in the *ATP12* gene of a patient with an ATP synthase decreased activity. Until now, nuclear defects of the ATP synthase complex have not been disclosed. Both multicopy and single copy plasmid constructs containing the human and yeast wild type *ATP12* gene, as well as the human mutation W94R and the yeast counterpart W103R were prepared. These constructs were introduced in a yeast host strain deprived of his *ATP12* function, and as such respiratory deficient. ATP synthase activity of individual yeast transformants was assayed by growth studies on a non-fermentable carbon source and by Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) stained for ATP synthase catalytic activity. WT plasmid constructs rescue the respiratory defect of a yeast *atp12* mutant strain. Growth on a non-fermentable carbon source was strongly impaired for the human W94R mutant, while the growth of the yeast W103R mutant strain was normal. These results were confirmed by BN-PAGE studies and measurements of the complex V activity of the different plasmid constructs in yeast cells. CONCLUSION : Our yeast complementation studies showed clearly that the human W94R mutant does not confer respiratory competence to an *atp12* yeast strain and is the cause of the complex V dysfunction in our patient.

P0700. Prediction of cis-acting splicing regulatory elements in the dystrophin gene

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We report a set of point mutations disrupting the correct pattern of dystrophin pre-mRNA splicing. We focused our attention on two types of events of peculiar interest for the definition of splicing regulatory elements (1) skipping-associated exonic mutations, (2) pseudoexons inclusion in mature transcripts. We have identified four different point mutations in the coding regions of the dystrophin gene leading to exon skipping. Such single base change are supposed to interfere with splicing by either disrupting Exonic Splicing Enhancer (ESE) elements or by creating negative-acting elements such as the Exonic Splicing Silencer (ESS) motifs. Moreover, we identified four cases of pseudoexon inclusion in the mature transcripts of Becker patients resulting (i) in three cases from an intronic variation which reinforces the strength of a pre-existing splice site, (ii) in one case from an intronic deletion in the vicinity of the pseudoexon suggesting the presence of a regulatory element in the deleted sequence. In order to correlate

the observed splicing defects with the presence of putative cis-acting splicing regulatory elements, we analysed the sequences of exons and pseudoexons for the presence of ESE, ESS, and ISE (intronic splicing enhancer) motifs. We used the ESE finder program and the RESCUE-ESE method to predict ESEs, and we searched for the recently described RESCUE-ISE predicted ISE hexamers and ESS motifs. Data from mutational analysis based on transcript studies allow to evaluate the reliability of splicing regulatory motifs prediction by in silico approaches, and contribute to identify new cis-acting regulatory elements.

P0701. The molecular diagnosis of Spinal Muscular Atrophy (SMA) in Romania

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Proximal forms of SMA are of 5q and non-5q type, the former being by far the most frequent. All cases of 5q SMA are determined by mutations of the SMN1 gene, the differences between clinical forms being explained by the influence of other modifying genes (e.g. NAIP). The vast majority of SMN1 gene mutations (95%) are localized in exons 7 and 8.

In our study, 24 clinically diagnosed children for SMA, and 16 parents, were included. The patients were classified according to clinical criteria (age of onset, clinical evolution) and EMG tests in: SMA1 (onset < 6 months of life): 19 patients; SMA2 (onset: 6-18 months): 3 patients and SMA3 (onset: >18 months of life): 2 patients. We have tested the presence of SMN1 exon 7 and 8 using the PCR-RFLP method and PCR for NAIP exon 5.

As shown in the table, the homozygous absence of exon 7 of SMN1 gene was observed in all patients with SMA1 and 2, but not in those with SMA3 and parents. The four patients with SMA1 and with complex deletions had an acute evolution. We note the high prevalence (47.4%) of SMN1 exon 8 deletion in our patients. Genetic counseling was offered to all couples having a child affected by SMA.

The distribution of mutations in SMA patients			
Homzygote deletion	SMA1	SMA2	SMA3
SMN1 exon 7	10	3	0
SMN1 exon 8	3	0	0
SMN1 exons 7 and 8	2	0	0
SMN1 exons 7, 8 and NAIP exon 5	4	0	0

P0702. Characterization of the molecular defects in the *ATP7B* gene in Wilson Disease patients from Bulgaria

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Wilson disease (WD) is an autosomal recessive disorder of the copper metabolism resulting from the absence or dysfunction of a copper transporting P-type ATPase, coded by the *ATP7B* gene. Pathogenesis of the disease is caused by accumulation of copper in liver, kidneys and brain. Approximately 240 mutations in the *ATP7B*-gene have been identified to date.

We screened all coding regions and exon / intron boundaries of *ATP7B*-gene in 77 Bulgarian families using single-strand conformational polymorphism (SSCP) followed by direct sequencing and characterized the molecular defect in 84% of WD chromosomes. In 65 of patients both mutations were identified. Fourteen different mutations including two non reported (D1279Y and P1352S) (Table 1) and nine polymorphisms within coding regions were observed. (Table 2).

Our results demonstrate that five mutation - H1069Q (52.60%), 2304-2305insC (9.74%), 3400delC (4.55%), G1341D (3.90%), and V12171218L (3.25%) are relatively common and account for 74% of WND alleles.

These data can be used to develop straightforward genetic testing in this population or in other countries composed of a genetically mixed population.

Table 1 . Mutations in the WND gene in Bulgarian patients

No	Mutation	Type	Exon	Alleles	Frequency %
1	R616Q	mutation	5	4	2.60
2	2298-2299insC	mutation	8	15	9.74
3	R778W	mutation	8	1	0.65
4	R778G	mutation	8	1	0.65
5	2530delA K844K	mutation	10	1	0.65
6	A874V	mutation	11	3	2
7	R969Q	mutation	13	2	1.30
8	A1003T	mutation	13	1	0.65
9	H1069Q	mutation	14	81	52.6

No	Name	Type	Exon	Alleles	Frequency %
10	3400delC	mutation	15	7	4.55
11	V12171218L	mutation	17	5	3.25
12	D1279Y*	mutation	18	1	0.65
13	G1341D	mutation	20	6	3.90
14	P1352S*	mutation	20	1	0.65
-	Known	-	-	130	84.42
-	Total alleles	-	-	154	100.00

Table 2 . Polymorphisms in the WND gene in Bulgarian patients

No	Name	Type	Exon
1	L456V	polymorphism	3
2	F764F	polymorphism	8
3	K832R	polymorphism	10
4	T991T	polymorphism	13
5	A1003A	polymorphism	13
6	L1015L	polymorphism	13
7	L1140A	polymorphism	16
8	K1260N	polymorphism	18
9	K1436K	polymorphism	21

P0703. Analysis of the JAG1 gene and clinical characterization of Polish patients with Alagille syndrome.

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Alagille syndrome (AGS) is an autosomal dominant disorder characterized by intrahepatic bile duct paucity and at least three of five main clinical anomalies: cholestasis, cardiac, skeletal and ocular abnormalities and a recognizable facial phenotype. AGS phenotype is caused by mutations of the JAG1 gene located on chromosome 20p12. The JAG1 gene encodes a ligand in the evolutionarily conserved Notch signaling pathway, taking part in cell fate determination and differentiation. In this study we present results of the JAG1 gene mutation analysis and clinical characterization of 42 Polish patients with clinically recognized AGS. Mutations were found in 23 patients. Twenty one different mutations, including 8 frameshift, 6 nonsense, 4 splice site and 3 missense mutations, were identified. Thirteen mutations are specific for Polish population. All mutations are localized in the extracellular domain of JAG1 protein. Most mutations lead to premature termination codons resulting either in creation of truncated proteins or in nonsense-mediated mRNA decay. Family studies revealed that the specific mutations were inherited in 6 out of 18 investigated cases. No apparent correlation between genotype and phenotype was observed. A considerable interindividual and intrafamilial variability of AGS features in investigated patients can be explained by existence of genetic modifiers.

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P0704. Polymorphisms in the CYP1A2 gene and Theophylline metabolism in Turkish patients with COPD

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Cytochrome P450 (CYP) 1A2 gene polymorphisms are thought to be involved in theophylline metabolism. We analyzed the effect of genetic polymorphisms -2964 (G/A) and -1569 (T/del) in the 5'-flanking region and 155 (T/G) and 731 (C/A) in the first intron of the CYP1A2 gene on theophylline metabolism in 100 Turkish patients with COPD (Chronic Obstructive Pulmonary Disease). Genetic polymorphisms were detected using PCR-RFLP technique. The frequency of the each genotype from four polymorphic sites in patients showed G/G= 93%, G/A=7%, A/A=0% for -2964 G/A position, T/T=2%, T/del=34%, del/del=64% for -1569 (T/del) position, T/T=92%, T/G=8%, G/G=0% for 155 (T/G) position, C/C=11%, C/A=58%, A/A=31% for 731 (C/A) position. Our results suggest that the "del" allele at position -1569 and "C" allele at position 731 in the gene may be causal factor which effect the CYP1A2 enzyme activity in chronic obstructive pulmonary disease.

P0705. Screening of the HFE gene mutations in patients with cryptogenic cirrhosis by PCR-RFLP technique

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Cirrhosis is usually accepted as cryptogenic only after an extensive evaluation has excluded recognizable etiologies. The prevalence of cryptogenic cirrhosis ranges from 5% to 30% of cirrhotic patients. We performed to screen the HFE gene mutations on a series of 15 patients (8 females and 7 males) with cryptogenic cirrhosis and 100 randomly selected healthy controls. Following DNA extraction from peripheral blood and PCR amplification, the PCR products were digested to determine C282Y, H63D and S65C mutations with RsaI, MboI and HinfI restriction enzymes, respectively. We detected a heterozygote H63D mutation in a patient with idiopathic cirrhosis. The data of patients and the healthy controls were compared for the HFE gene mutations and discussed with literature. Although number of the patients is a few, our results suggests that HFE gene mutations should be screened in patients with cryptogenic cirrhosis.

P0706. Relative prevalence of GJB2 gene mutations in autosomal recessive nonsyndromic deaf population in Kerman province of Iran

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Mutations in the GJB2 gene (at the DFNB1 locus) encoding the gap junction protein connexin 26, are responsible for 50% to 70% of autosomal recessive non-syndromic hearing loss (ARNSHL) in many populations of European descent. To assess the importance of GJB2 mutations in the Kerman deaf population in southeast of Iran, we screened 65 probands (130 chromosomes) with ARNSHL. Genetic testing began with an allele specific polymerase chain reaction (ASPCR) assay to screen patients for the 35delG mutation (the most common mutation in many world populations). Patients heterozygous or negative for the 35delG mutation were screened by DHPLC and sequencing for other GJB2 mutations. GJB2 mutations were detected in 12 (9.2%) chromosomes. Homozygosity or compound heterozygosity for GJB2 deafness causing allele variants were found in 5 patients (7.7%). Allele variants found were R127H (4 alleles), 35delG (3 alleles), W24X (2 alleles), delE120 (2 alleles), R143W (1 allele) and, V153I (5 alleles). V153I is a benign polymorphism. The dominant most frequent mutation was R127H (33.3%) rather than 35delG mutation. Since the frequency of GJB2 mutations is much

lower in this province comparing to the rest of Iran and other parts of the world, we propose that other genes may play a significant role in the ARNSHL genetic load in Kerman province.

P0707. Two different-sized D4Z4 repeat within a FSHD family with an high clinical variability

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy characterized by progressive atrophy and weakness of the facial, shoulder, upper and lower limbs with high inter- and intrafamilial clinical variability. FSHD is associated with contractions of the polymorphic D4Z4 repeat on chromosome 4qter. A rough and inverse correlation has been observed between the severity and age at onset of the disease and the residual repeat unit number. We report on a family with a significant intrafamilial clinical variability and two different-sized D4Z4 repeats. Indeed, 4/10 patients presented a predominant scapular form and 6/10 patients presented a predominant peroneal form. The mean age at onset of symptoms was of 38 years, but highly varied from 8 years to 58 years. We did not find any correlation between the length of the repeat unit and the age of onset or the predominant location of the muscle weakness. Hypotheses in order to explain the two different-sized D4Z4 repeats included: 1) a common ancestor compound heterozygote for two different-sized D4Z4 alleles, 2) a common ancestor with de novo somatic mosaicism with two different-sized D4Z4 repeats on the same allele secondary to gene conversion with intrachromosomal crossover, 3) an affected individual with an inherited D4Z4 contraction and acquired somatic mosaicism with two different-sized D4Z4 repeats on the same allele secondary to gene conversion without intrachromosomal crossover. Interestingly, the second mechanism has been recently reported in 3 cases (Lemmers, AJHG 2004, 75: 44-53), indicating that gene conversion might not be a rare event in FSHD.

P0708. A novel mutation in the ARS (component B) gene encoding SLURP-1 in a Turkish family with Mal de Meleda

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Mal de Meleda (MDM) is an autosomal recessive, transgressive palmoplantar keratoderma (PPK) associated with keratotic skin lesions, perioral erythema, brachydactyly and nail abnormalities. The ARS (component B) gene has been mapped on chromosome 8qter and mutations have been implicated in MDM. The gene contains 3 exons and encodes the protein named secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related protein-1 (SLURP-1). The small Turkish family with MDM has been analysed for the ARS gene mutation(s). DNAs were extracted from the peripheral blood of the family members and the ARS gene were sequenced by the automated DNA sequencer (ABI Prism 310). The mutation was detected in the 2nd exon of the gene. The mutation was changing of cysteine residue to premature stop codon at the position 43. The son diagnosed as the MDM was homozygote for the mutation whereas the parents were heterozygote

P0709. Multiplex SNP genotyping using Luminex bead arrays

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An economical multiparameter assay based on Fluorescent Bead Arrays (FBA) and the Oligonucleotide Ligation Assay (OLA) is described, which allows Single Nucleotide Polymorphism (SNP) genotyping of PCR amplified samples. The method is based on the Luminex LabMAP technology using microsphere beads available in one hundred different fluorescent color tones of red and infrared. These microspheres serve as solid phase for the SNP genotyping reaction. Each bead species is coupled to a detection oligonucleotide carrying the discriminating base at the 3' position. After amplification of the relevant gene fragment a

trimeric hybridization complex is formed consisting of one strand of the PCR product on one hand and the bead-coupled discriminating oligonucleotide and an adjacent 3' biotinylated signal oligonucleotide on the other hand. Detection and signal oligonucleotide meet at the site of mutation due their complementarity to the sequence up and downstream of the test locus. Subsequently, a ligase is added which ligates the two nucleotides only when all nucleotides at the junction site are basepaired. Mismatched DNA fragments are not ligated. The detection of the ligated DNA fragments is accomplished by the read-out of the biotin bound streptavidine-phycoerythrine conjugate within the Luminex analyzer.

This highly accurate and sensitive no-wash assay can be used for economical genotyping reactions in the field of routine diagnostics as well as in research. Due to the high flexibility of the FBA up to fifty SNPs can be analyzed simultaneously in a single sample.

P0710. Complex genetic analysis of the AZF region, AR and CFTR on patients with various forms of male infertility

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Y-chromosome microdeletions (AZF region), mutations of *CFTR* and alleles CAG_{>26} of the AR are the most common genetic factors for male infertility. AZF loci microdeletions are responsible for azoospermia and severe or moderate oligospermia. These diseases are also associated with certain allelic variants of the AR CAG-repeat. The risk of pathology in men with CAG_{>26} is 4 times higher than the population average. Congenital absence of the vas deferens in some cases is caused by a combination of mutations in *CFTR*.

The purpose of this study is the complex molecular analysis of AZFa, b, c loci, AR and *CFTR* in Russian patients with various forms of male infertility. We have investigated 76 DNA samples from unrelated patients of Russian origin. Y-chromosome microdeletions were analyzed using 6 markers. We also determined the length of the AR CAG-repeat, the presence of delF508 and allelic variants IVS8(T)n in *CFTR*.

We detected AZF deletions in 8 patients with oligo- or azoospermia (10.5 % of cases). AR alleles CAG_{>26} were observed in 3 cases. The patient with absence of the vas deferens had a *CFTR* genotype delF508 / 5T. Two heterozygous carriers of the delF508 and 5 carriers of a 5T mutation were revealed. Altogether, screening has revealed mutations and predisposing allelic variants in 23 (30.2 % of cases) patients with male infertility. Detection of heterozygous carriers of *CFTR* mutations provides differentiation of the infertility and furthermore prevents cystic fibrosis.

P0711. Evaluation of Association Between Glutathione S-Transferase M1 and T1 Genotypes and Myocardial Infarction

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Myocardial infarction (MI) is the most frequent causes of mortality and morbidity. Heredity is the most important risk factor for developing of MI. Association between MI and genetic basis have been not established sufficiently. We aimed to evaluate distribution of glutathione S-transferase (GST-M1) and (GST-T1) genotypes in patients with MI, for investigated whether polymorphisms in these genes influence risk of coronary artery disease. The study population consist of 153 patients with MI admitted our Hospital to seek medical attention and 131 healthy persons as a control group. DNA was extracted from whole blood by using DNA extraction method and the GST-M1 and GST-T1 polymorphism were determined using a multiplex PCR technique. We evaluated relation between coronary artery diseases and distribution of GST-M1 and GST-T1 polymorphisms.

P0712. Rett syndrome - MECP2 mutations in Polish patients with classic form and variants of the disease.

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Rett syndrome (RTT, MIM#312750) is a progressive neurological disorder affecting almost exclusively females. Mental retardation, loss of acquired skills, stereotypical hand movements, seizures, scoliosis and autonomic dysfunction are the common clinical features appearing after a few months of almost normal development. Besides the classic form of RTT, five distinct variants have been described. The prevalence of the disease is estimated to be 1 in 10000 - 15000 female births. RTT results from mutations in *MECP2* gene in chromosome Xq28 coding methyl-CpG-binding protein 2 (MECP2). To date more than 300 various *MECP2* mutations have been found.

The aim of our study was to investigate molecular basis of Rett syndrome in Polish population. *MECP2* gene mutation analysis was performed in 123 female patients with classic form and variants of RTT. Genomic DNA was extracted from lymphocytes, and coding region of *MECP2A* isoform was amplified with 8 primer pairs and analyzed by SSCP analysis. Amplicons with shifted mobility were subsequently sequenced. Twenty five different *MECP2* mutations were found in 45 probands from all analyzed families (37%), including 9 missense mutations, 4 nonsense mutations and 12 frameshift mutations. Nine mutations (K305Q, E472Q, c.329delA, c.506_519dup14, c.697_701del5, c.883delT, c.975_1186del212ins18, c.1162_1178del17, c.1353_1354insA) were specific for Polish patients. Almost all mutations are clustered in exon 4, mainly in regions coding methyl-binding domain (MBD), transcription repression domain (TRD), and C-terminal part of MECP2 protein. Seven recurrent mutations (R106W, T158M, R168X, R255X, R270X, R294X, R306C) account for 54% of all mutated alleles.

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P0713. Calcium-Sensing receptor gene A986S polymorphism and bone mass in hypertensive women.

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Only a few studies have analyzed the relationship between bone mass and hypertension, finding an inverse relationship between bone mineral density and hypercalciuria (the most frequent disorder of calcium metabolism in hypertension). The function of the Calcium Sensing receptor is to maintain the serum calcium concentration within a narrow physiological range. Activating and inactivating mutations of the gene causes hypercalcemia and hypocalcemia respectively. Allele S of A986S polymorphism, is associated with higher levels of calcium and lower calciuria.

CaSR acts exerting an inhibitory effect on the Na-Cl Cotransporter (NCCT) of the distal convoluted tubule.

To assess the effect of Calcium-Sensing receptor A986S polymorphism, in 48 hypertensive women with mild-to-moderate hypertension, we determined: Calcium, Phosphorus, Magnesium, PTH, 25(OH) vitamin D, 1,25(OH)₂ vitamin D, osteocalcin, deoxypyridinoline in urine, 24-hour urine calcium and bone densitometry of the lumbar spine and studied by PCR Calcium Sensing receptor gene A986S polymorphism.

Genotype frequency was 69% for AA, 27% for AS and 4% for SS, with an allelic frequency of 82% for allele A and 18% for allele S.

There wasn't differences between patients with and without allele S in calcium metabolism parameters, bone remodelling markers and bone mineral density, except lower levels of phosphorus in patients without allele S (3.5+- 0.5 vs 4.0 +/- 0.4; p=0.034).

Conclusions: The Calcium Sensing Receptor A986S Polymorphism have no clinical significance in post-menopausal hypertensive women.

P0714. Spectrum of DHCR7 gene mutation in 48 patients with SLOS and carrier frequency of the disease in Poland.

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations in the DHCR7 gene. Forty eight Polish patients with SLOS underwent mutation analysis. In total we identified 15 DHCR7 mutations. The mutation frequencies in Polish patients were significantly different from those observed in Western European populations. Two mutations, W151X (35/96 alleles, 38,2%) and V326L (26/96 alleles, 27,0%) accounted for 65,2% of all observed in our cohort. By comparing clinical severity scores, and the biochemical and molecular data a genotype-phenotype correlation was attempted. Mild phenotypes are correlated with mutations affecting the putative transmembrane domains TM1-TM6 or CT regions and severe phenotypes with mutations localized in TM7 and 4L region. The most severe and lethal cases were related with genotype 0/0. The phenotypic differences of patients with the same genotype suggest that severity of the disease may be affected by other factors. The disease is most prevalent among populations of Central Europe (Czech Republic 1:10000, Slovakia 1:15000-1:20000). Published data indicated that the incidence of SLOS may be as high as 1:1590 -1:13500 in USA (state of Oregon) population of European ancestry while in general populations of the United Kingdom and the United States of America the incidence is estimated as 1:60000. The observations of Polish groups indicate that the disease carrier rate is approximately 1 in 27 and SLOS incidence is estimated as 1:2646 to 1: 4065.

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P0715. Analysis of telomerase activity in cervical samples as a diagnostic biomarker of cervical dysplasia.

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Telomerase activity and human telomerase reverse transcriptase (hTERT) mRNA expression were investigated in cervical specimens and were correlated with cytologic findings and the presence of HPV infection. Telomerase activity was evaluated by the telomeric repeat protocol assay (TRAP) and hTERT mRNA expression by RT-PCR. HPV DNA was detected by PCR, as well as restriction endonuclease digestion. HPV DNA was detected in all 82 specimens with abnormal cytologic findings and in 4/34 normal samples. Low grade squamous intraepithelial lesions (LGSILs) were present in 74/82 specimens (90.2%) and high grade squamous intraepithelial lesions (HGSILs) in 8/82 (9.75%). Seven of the 8 HGSIL (87.5%) and 26/74 LGSIL (35.1%) specimens were hTERT positive, while all normal specimens were hTERT mRNA negative. Telomerase activity was detected in 21/74 (28.4%) LGSIL/ASCUS and in 5/8 (62.5%) HGSIL samples. A correlation was observed between telomerase activity, hTERT mRNA expression and high risk HPV infection in HGSIL samples (p<0.001). Telomerase activity assessment in cervical smears showed sensitivity and specificity for HGSILs 62.5% and 80.5% respectively, while hTERT mRNA expression assessment showed 87.5% sensitivity and 86% specificity for HGSIL. Based on the above telomerase assessment values it is suggested that the telomerase system might be an appropriate diagnostic marker for cytology. However, the final evaluation must rely on a combination of all available test assessment data and clinical diagnosis.

P0716. Mutation detection in Iranian patients affected with Cystic fibrosis

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Cystic fibrosis (CF), a common autosomal recessive disease, results from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Since the isolation of this gene, almost 1300 mutations and a large number of polymorphisms have been discovered. It has been recognized as the most lethal genetic disease, CF often causes death in children. The frequency and the spectrum of CFTR

gene mutations vary in different populations.

The main object of this study was to determine the type of CF mutations in the Iranian population and to perform prenatal diagnosis. This study included 14 couples referred to our center. Using a test panel of 31 known European mutations, we analyzed samples from each individual by multiplex OLA-PCR and ABI Prism 3100 genetics assays. At this point, for patients in whom we detected no mutation, we performed SSCP and DNA sequencing. We identified the following six mutations in 30 of 54 CF chromosomes: dF508 (n=12), G542X (n=6), S466X (n=4), R1070Q (n=4), N1303K (n=2), 4005-1G->A (n=2). The overall detection rate was 41%. For the purpose of first trimester prenatal diagnosis, we performed direct mutation analysis on fetuses from four CF families. The results showed that all four fetuses were homozygous.

P0717. The R369C mutation in the CBS gene: characterization and frequency

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Cystathionine beta-synthase (CBS) deficiency is the most common cause of homocystinuria. More than 130 pathogenic mutations in CBS gene were detected on ~550 homocystinuric alleles. One of them, the R369C mutation was described as a very frequent variant among newborns in Norway. In contrast, only four patient alleles carrying this mutation were detected worldwide. As the protein carrying R369C was not yet characterized, we expressed this mutant by prokaryotic expression system and we examined the prevalence among newborns.

To characterize the R369C mutation, we expressed R369C mutant in E.coli. Under physiological conditions (37°C) the mutant enzyme had no detectable activity, and form aggregates. However, the addition of pyridoxal-5-phosphate (PLP) increased the enzyme activity to 10% of wild type enzyme. In addition, the PLP saturated enzyme was also activated by S-adenosylmethionine (SAM) similarly like wild type CBS. These data suggests that R369C is indeed pathogenic mutation, although its activity may be stimulated.

We analyzed 600 healthy newborns for the presence of R369C mutation. The heterozygosity for the mutation was observed in 6/600 samples, corresponding to expected incidence of homozygotes to 1:40.000 (95%CI 1:8.000-1:295.000). These data confirmed that R369C frequency in another European populations is also very high, supposedly leading to high frequency of homozygotes.

To conclude, the R369C mutation is a pathogenic variant which may be activated by PLP and SAM and heterozygosity for this variant may confers a mild clinical phenotype which may be missed by existing diagnostic procedure.

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P0718. Mutations and Fenotype/Genotype Relations in 69 FMF Patients

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Familial Mediterranean Fever (FMF) is an inherited disorder characterized by episodes of recurrent fever, arthritis and pleuritis as well as development of amyloidosis.

In this study, the frequency of M694V, M694I, V726A, M680I and E148Q mutations and phenotype-genotype correlations among 69 patients with clinical diagnosis of FMF have been tested. The correlation between genetic analysis and clinical findings were evaluated.

Mutations were present in 53(76.8%) of 69 cases enrolled into study. Of 106 alleles 50(47.2%) were M694V, 9(8.5%) were V726A, 3(2.8%) were M694I, 9(8.5%) were E148Q and 9(8.5%) were M680I mutations.

In clinical evaluation according to Tel Hashomer criteria, the positive major and minor criteria for patients with homozygote, heterozygote and patients with no mutations were shown in table.

Table. Total numbers of criteria stratified according to mutations

Genotype	Tel Hashomer Criteria Total number criteria (criteria per patient)	
	Major	Minor
Homozygote Mutation (n=11, 20.5%)	17 (1.55)	14 (1.3)
Heterozygote Mutation (n=42, 56.3%)	45 (1.07)	52 (1.24)
No Mutation (n=16, 23.2%)	16 (1)	15 (0.94)

Among all cases, the frequency of Tel Hashomer criteria in homozygote mutations group was greater than other groups. Although the frequency of criteria in 16 patients who had none of these five mutations was less than the other two groups, the number of major and minor criteria per patient was not as low as to decide not to examine mutations.

P0719. Exon repetition mutations in Dygge Melchior Clausen syndrome

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Dygge Melchior Clausen syndrome (DMC OMIM 223800) is an autosomal recessive osteochondrodysplasia characterised by short stature with a short trunk, multiple bony abnormalities and mental retardation. The DMC gene on chromosome 18q21 encodes a protein product, dymeclien with no homology to any known protein. To better characterise the DMC gene we performed RT-PCR of dymeclien cDNA in subjects from 2 kindreds and detected aberrantly sized products compared to control samples. Sequencing revealed a duplication of exon 2 and repetition of 4 copies of exon 14 respectively. Fluorescent dosage PCR and Southern blot analysis of genomic DNA were consistent with these findings. Southern blot analysis of the exon 14 repetition allowed us to further delineate the region of duplication and studies underway are continuing to narrow down this region. Both mutations lead to a frameshift introducing a premature stop codon with presumed loss of protein as the pathogenetic mechanism.

P0720. Three novel mutations in the second exon of CYP1B1 in Iranian Primary Congenital Glaucoma patients

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Primary Congenital Glaucoma (PCG) is an important cause of childhood blindness in Iran. We aimed to determine the proportion of Iranian patients afflicted with PCG whose disease is due to mutations in the cytochrome P450 1B1 (CYP1B1) gene at locus GLC3A. Mutations in both alleles of the gene result in the PCG phenotype. The gene has three exons, two of which are coding. Most reported mutations in the gene are located in the coding exons. In this part of the study, mutations were sought in exon 2 which is the first coding exon and has approximately 1000 bp.

Sixty six chromosomes from thirty three unrelated Iranian patients afflicted with glaucoma at birth or before the age of 3 years old were studied. The second exon and neighboring intronic sequences of CYP1B1 was amplified from DNA of peripheral blood in two overlapping PCR reactions. PCR products were sequenced by the di-deoxynucleotide termination protocol using fluorescent labeled nucleotides. Sequence data and chromatograms were analyzed by the Sequencher software.

Twenty alleles (30%) among the sixty six chromosomes studied had putative disease causing mutations in exon 2 of the CYP1B1 gene. Five distinct mutations were detected, three of which are novel. The new mutations included a nine base pair duplication (c.N1166dupGCACTTCA), a single nucleotide deletion (c.N553del A), and a missense mutation (c.N887G>A; p.E173K). Additionally, six putative polymorphisms were identified. As reported for other populations, mutations in the second exon were less frequent than mutations in the third exon.

P0721. Novel *CLCN5* gene mutations in Spanish families with Dent disease

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Dent disease is an X-linked renal tubular disorder characterized by low-molecular-weight proteinuria, hypercalciuria, nephrolithiasis, nephrocalcinosis and renal failure. This disease is associated with mutations in the *CLCN5* gene encoding the voltage-gated CIC-5 chloride channel that participates in the acidification of proximal tubule endosomes. Our goal was to identify and characterize *CLCN5* mutations in Spanish patients with Dent disease.

Blood DNA from 11 unrelated patients and relatives was used for PCR amplification. The *CLCN5* coding exons and their intronic flanking regions were analysed by automatic sequencing. Defects in pre-mRNA splicing were analysed in blood samples by RT-PCR.

We identified 8 new mutations: 2 nonsense (R467X, R704X), 2 missense (C219R, W547G), 1 frameshift (976delG), and 3 splicing (IVS2-2A>G, IVS3-6A>T, IVS9-30C>T). In some cases, the mutations were shown to co-segregate with the disease. Analysis of RNA from the patient with the mutation in intron 2 acceptor splice site showed an aberrant *CLCN5* pre-mRNA splicing. A similar analysis of the other two intronic mutations, located at the polypyrimidine tract and near the branch site, respectively, showed no splicing defect. We also found that the previously identified *Alu* insertion in the coding region of *CLCN5* led to a splicing defect that skipped exon 11.

These mutations predict structurally significant alterations of the CIC-5 protein and are likely to result in loss of chloride channel function. The absence of *CLCN5* mutations in 2 patients indicates that variants in other genes could be associated with Dent disease.

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P0722. Molecular diagnosis of HED : a five years experience

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Hypohidrotic ectodermal dysplasia (HED) is the most common form of over 150 different ectodermal dysplasias. This syndrome is characterized by the triade : hypohidrosis, hypodontia and hypotrichosis. The X linked form, caused by a mutation in the *ED1* gene, represents the majority of HED. However a minority of HED is inherited as a dominant or recessive autosomal trait. These last result from mutations in at least two genes *EDAR* and *EDARADD*.

We carried out a mutational analysis of *ED1* gene in 145 unrelated HED families or sporadic cases between 2000 and 2005,

EDAR gene in 37 of 46 unrelated subjects with no mutation in *ED1*, *EDARADD* gene in 28 unrelated subjects neither *ED1* nor *EDAR* HED.

Our investigations allowed us to better assess the implication of the three loci in HED :

66% of HED were caused by mutation in *ED1* gene (75% in affected males versus 25% in affected females)

about 25% of non *ED1* HED were related to *EDAR* (2/3 males, 1/3 females)

and only one case presented a mutation in *EDARADD* gene.

Review of clinical features showed no obvious phenotype/genotype correlation. We can only observe more sporadic cases in *EDAR* related HED.

Our study supports a molecular diagnostic strategy based only on the implication frequency of the different genes because of the large variability of clinical expression and the smaller size of most of the families making linkage analysis difficult. A exception should be made for a consanguineous family where *EDAR* and *EDARADD* should be tested first.

P0723. Quantification of cell-free fetal DNA in the maternal circulation and its correlation with clinical outcome of pregnancy

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Objective: Recently, multiple studies has demonstrated the existence of cell-free fetal DNA in the blood of pregnant women. An elevation of DNA concentration in maternal plasma has been determined in various cases of pregnancy complications (preeclampsia, preterm labor, polyhydramnion etc.) or cytogenetic abnormalities (fetal aneuploidy). In our study, the concentration of cell-free fetal DNA in maternal plasma has been measured during all trimesters of pregnancy and the results were compared with clinical data.

Methods: Fetal SRY-specific DNA quantification was carried out by real-time PCR method on the ABI PRISM 5700 and 7000 machines using the MGB probe and a couple of primers. Cell-free DNA isolated from plasma of 200 pregnant women were tested.

Results: We have found statistically significant elevation of fetal DNA in maternal plasma in the 3rd trimester both in the twin pregnancies with two male fetuses ($p = 0.037$) and with one male and one female fetus ($p = 0.033$), resp. We have seen even higher elevation of fetal DNA concentration ($p = 0.096$) for the IUGR and hypertension compared with the normal pregnancy women sample. No significant elevation of cell-free fetal DNA in maternal plasma has been demonstrated in females with APA (antiphospholipide) syndrome ($p = 0.511$).

Conclusion: Quantification of cell-free DNA in maternal plasma is a potentially useful marker for monitoring placental abnormalities, but further studies concerning the mechanisms of its transfer, turnover and clearance in maternal plasma are needed.

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P0724. Hailey-Hailey disease as an exemplary orthodisease

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The term orthodisease has recently been introduced to define human diseases in which the pathogenic gene has orthologs in model organism genomes. In this presentation Hailey-Hailey disease (HHD), a blistering skin disorder due to haploinsufficiency of ATP2C1 is reviewed as an orthodisease from a *Saccharomyces cerevisiae* perspective. ATP2C1 encodes the human secretory pathway Ca2+/Mn2+ ATPase hSPCA1 and is orthologous to the PMR1 gene initially identified in *S. cerevisiae*. hSPCA1 fully complements PMR1 deficiency in yeast and pmr1^Δ *S. cerevisiae* has proved to be a valuable tool to screen ATP2C1 mutations and address potential pathogenic/pharmacologic mechanisms in Hailey-Hailey disease. We have recently found that a PMR1 deficient yeast is less reactive to increasing levels of extracellular calcium similarly as HHD keratinocytes are. Additionally calcineurin inhibition induced tolerance to high levels of extracellular calcium for a pmr1^Δ strain suggesting a possible direct beneficial cellular mechanism of calcineurin inhibition in the treatment of HHD. Our findings lend further evidence that this human skin disorder is an ideal example of an orthodisease.

P0725. The novel 312-326del14 mutation of the connexin 26 gene is the second common cause of non-syndromic sensorineural hearing loss in patients with deafness from Bashkortostan

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Several studies have reported that mutations in the GJB2 gene are a common cause of non-syndromic hearing impairment. More than 80 different mutations of the GJB2 gene have been 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 identified in several populations including the 35delG mutation in Caucasians (70% of all GJB2 pathogenic alleles), the 167delT

allele in Ashkenazi Jews (carrier rate is 3-4%), the 235delC allele in Asian populations (80% pathogenic GJB2 alleles among Japanese). We report the identification of a novel 14bp deletion of the coding region GJB2 gene in a Russian family with autosomal dominant neurosensory deafness. This mutation results in the deletion of five amino acids, including K105, an amino acid residue, which is highly conserved throughout evolution. The 14bp deletion co-segregates with the diseases phenotype in these patients family, but is not found in unaffected relatives or 50 normal individuals. The 312-326del14 mutation is believed to be pathological: first, because of its location and conservation and, second, because no change has been observed in a series of normal control. 312-326del14 mutation of the connexin 26 gene accounts for up to 3% of pathogenic GJB2 alleles among patients with nonsyndromic hearing impairment from Bashkortostan. So, the 312-326del14 mutation in GJB2 is the second most frequent cause of non-syndromic hearing impairment in our region.

P0726. At least 38% of Iranian Primary Congenital Glaucoma patients carry two mutated alleles of CYP1B1

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Glaucoma is a heterogeneous group of optic neuropathies characterized by degeneration of the optic nerve, usually associated with elevated intraocular pressure. Primary congenital glaucoma (PCG), which becomes apparent at birth or before the age of three, is a major cause of childhood blindness. We aimed to determine the proportion of Iranian PCG patients whose disease is due to mutations in the cytochrome P4501B1 (CYP1B1) gene. Mutations in both alleles of the gene result in the PCG phenotype. The gene has three exons, two of which are coding. In this part of the study, mutations were sought in exon 3 which is the second coding exon and has approximately 600 bp.

One hundred thirty six chromosomes from 68 unrelated Iranian PCG patients were studied. The third exon of CYP1B1 was amplified from DNA of peripheral blood by PCR. The amplified products were sequenced by the di-deoxynucleotide termination protocol. Sequence data and chromatograms were analyzed by the Sequencher software. Fifty four alleles (40 %) among the 136 chromosomes studied had putative disease causing mutations in exon 3 of the CYP1B1 gene. Seven distinct mutations were detected, two of which are novel. The new mutations were c.N1472C>T (p.R368C) and c.N1565A>G (p.I399V). Additionally, two putative polymorphisms were identified. Among seventy nine Iranian PCG patients in whom exon 2 and/or exon 3 of the CYP1B1 gene were sequenced, thirty carried two putative disease causing mutations in the gene, indicating that mutations in CYP1B1 account for disease in at least 38% of Iranian PCG patients.

P0727. Novel 1308delT mutation of the RPE65 gene in patients with nonsyndromic retinitis pigmentosa from Bashkortostan

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Retinitis pigmentosa (RP) is a group of inherited disorders in which abnormalities of the photoreceptors or the retinal pigment epithelium of the retina lead to progressive visual loss. RP is a clinically and genetically heterogeneous group of retinal degenerative diseases. The prevalence of RP is 19 to 27 per 100,000 in general population. At least 40 different genes or loci are known to cause nonsyndromic RP. RPE65 has a crucial role in the metabolism of vitamin A in the visual cycle. Gu et al. (1997) estimated that RPE65 mutations account for approximately 5% of autosomal recessive childhood-onset severe retinal dystrophy. We studied patients with RP and their relatives from Bashkortostan. We examined all 14 exons of RPE65 gene in 119 unrelated patients with RP and 77 unaffected individuals by single-strand conformation polymorphism analysis (SSCP) and direct sequencing. Patients were examined clinically and with visual function tests. We report the identification of a novel 1bp deletion (1308delT) of

the 12 exon RPE65 gene in 6 unrelated patients with sporadic form of RP from Bashkortostan. All these patients carried 1308delT mutation in homozygous form and have similar fundus appearances characterised as a maculodystrophy. Frequency of 1308delT mutation of the RPE65 gene among the patients with RP from Bashkortostan is 0.05 on mutant chromosomes. 1308delT of RPE65 gene heterozygous carriers were not identified in 77 healthy controls from Bashkortostan.

Identification of the molecular defects underlying retinal degeneration will allow clinicians to establish more accurate diagnoses and prospects for prenatal diagnostic.

P0728. Sulfatase activities are regulated by the interaction of the sulfatase modifying factor 1 (SUMF1) with SUMF2

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Sulfatases undergo a unique post-translational modification that converts a highly conserved cysteine located within their active site into formylglycine. This modification is necessary for sulfatase catalytic activity, and it is promoted by the protein product of *SUMF1*, the gene mutated in Multiple Sulfatase Deficiency (MSD). A paralogous gene, *SUMF2*, was discovered because of its sequence similarity to *SUMF1*. Both immunofluorescence and co-immunoprecipitation experiments show that *SUMF2* co-localizes with *SUMF1* within the endoplasmic reticulum and that the two proteins form cysteine-mediated heterodimers. Furthermore, we show that *SUMF1* and *SUMF2* are also able to form homodimers. We have previously demonstrated that co-transfection of *SUMF1* with sulfatase cDNAs greatly enhances the activities of over-expressed sulfatases. Now we obtained data demonstrating that *SUMF2* inhibits the enhancing effect of *SUMF1* on sulfatases. These results suggest that *SUMF1*-*SUMF2* interaction represents an additional level of control of the sulfatase activities.

P0729. Diagnosis of lysosomal storage diseases in Romanian patients

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Lysosomal storage diseases represent an important group among inherited metabolic disorders. To date, more than 40 different lysosomal storage diseases have been identified, characterised by a deficiency of a lysosomal protein. We report the experience of the Romanian centre for the diagnosis of lysosomal storage diseases. 106 suspects originating from all regions of Romania were referred to our centre for further evaluation and specific diagnosis. Initial work-up was based on clinical and radiological evaluation. Lysosomal enzyme assays in peripheral leukocytes were performed according to standard techniques and indicated specific deficiencies in 46 patients (43.4%). Gaucher disease was diagnosed in 34 patients, followed by the mucopolysaccharidoses and mucolipidoses patients (5 mucopolysaccharidosis III B patients, one mucopolysaccharidosis VII patient and one mucolipidosis II patient) and the group of sphingolipidoses - other than Gaucher disease (2 GM1 gangliosidosis patients, one GM2 gangliosidosis, one metachromatic leukodystrophy and one Fabry disease patient). Frequent mutations in the glucocerebrosidase gene were screened by PCR-RFLP and indicated an important prevalence of the N370S allele (56.45%) followed by the L444P (20.97%) and recNc1 (4.84%) alleles. Sporadic, unknown mutations accounted for 17.74% of the mutant alleles in Gaucher disease patients. Genotype-phenotype correlations in Romanian Gaucher disease patients were similar to those reported in other Caucasian populations, but also indicated specific characteristics. We conclude that lysosomal storage diseases and especially Gaucher disease may represent an important pathology in our population, and that specific laboratory diagnosis and follow-up is the key step in the accurate management of these patients.

P0730. Lack of association of TaqlB polymorphism in the CETP gene with HDL cholesterol levels and the risk of CAD in Turkish population.

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Genetic variants at the cholesteryl ester transfer protein (CETP) locus have been associated with CETP activity. Several studies have reported that the CETP TaqlB polymorphism is associated with HDL cholesterol levels and the risk of coronary artery disease (CAD), but the results are inconsistent. We have examined allele frequencies and lipid associations for the common CETP TaqlB polymorphism in a sample of 317 population controls and 185 angiographically evaluated patients with CAD. The prevalence of the TaqlB2 allele was 0.42 in population controls and 0.4 in CAD (CETP distribution does not differ from H-W p=0.98 and p=0.62). After adjustment for study age, sex, body mass index (BMI), diabetes, activity, alcohol and smoking, TaqlB genotype was not associated with HDL cholesterol levels and the risk of CAD (p=0.46).

The CETP TaqlB polymorphism did not show a significant interaction with other risk factors in influencing CAD risk. Our findings do not support the hypothesis that a genetic variant resulting in lowered CETP activity is associated with reduced risk of coronary atherosclerosis.

P0731. Clinical Findings and Mutation Analysis of Capillary Morphogenesis Protein 2 (CMG2) Gene in Two Sibling Patients with Juvenile Hyaline Fibromatosis

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Juvenile hyaline fibromatosis (JHF) is an autosomal recessive trait that usually presents skin lesions, often on the scalp and ears, around the nose and on the hands, which require recurrent excision, in the first years of life. Diagnosis is confirmed by histological examination of the skin, which typically shows the presence of hyaline deposition in the dermis.

we present a 12 year follow-up of clinical findings in two siblings (one male and one female) with JHF in a Turkish family. Both patients have undergone eleven operations for skin tumors and gingival hypertrophy. In the latest examination, they had numerous subcutaneous nodules on the scalp, face and fingers, but no gingival hypertrophy or joint contractures. The male patient had big tumors on his ears and osteolytic bone lesions on his hands, more than in his sister.

In molecular genetic analysis, we undertook mutation screening of the capillary morphogenesis protein 2 (CMG2) gene, which was mapped to chromosome 4q21, in members of the family. By direct sequencing, we have identified mutations in the probands and both parents, who have a consanguineous marriage. Both sibling patients were homozygous for a 25816 TT mutation in IVS17, and both parents were heterozygous (GT) for this variant. The elder brother had another new heterozygous mutation, insG at 2497 in IVS7 while he was heterozygous (GT) for the IVS17 mutation of CMG2. These mutations have not been reported previously.

P0732. Molecular Diagnosis of X-CGD in Iran

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Chronic Granulomatous Disease (CGD) is an inherited phagocytic disorder caused by mutations in NADPH oxidase subunits. Patients with CGD have life-threatening bacterial and fungal infections. It has been reported that most common presentation of the disease is caused by mutation in CYBB gene located on the X chromosome (Xp21.1), coding for gp91^{phox}. Diagnosis of CGD is made by demonstrating absent or markedly reduced oxidase activity in stimulated neutrophils. In order to facilitate final diagnosis of CGD and mutation analysis of CYBB gene, we have developed molecular diagnosis of the disease.

We have done CGD screening by NBT slide test, quantitative NBT and flowcytometric analysis using DHR123 for the patients and their family.

Since characterization of mutation is very important for detection of carriers and prenatal diagnosis, genomic DNA from 10 unrelated male patients with X-linked CGD was isolated and CYBB exons were amplified. PCR products were subjected to SSCP analysis on silver stained polyacrylamide gels.

Our CGD screening result over 24 patients shows that X-CGD is as common as autosomal recessive CGD in Iran, which was roughly 50%. Sequence analysis for PCR products related to abnormal migration on SSCP gels for the first seven exons of CYBB gene from X-CGD patients didn't show any mutation up to now.

We are now continuing sequencing by ABI prism BigDye terminators. During the course of study any new mutation will be reported to Human Mutation Data Bank.

P0733. Screening of mutations in ENG and ALK1 genes in hereditary hemorrhagic telangiectasia patients using denaturing high performance liquid chromatography

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Background: Our previous molecular studies of HHT patients was based on heteroduplex analysis, which allows a rapid screening at low cost but has not a very high sensibility, needs the use of radioactivity and remains a manual technique. Methods: Our protocol was based on touchdown PCR using the Optimase Taq and the Wave system (Transgenomic). The 90 different mutations, previously found in our patients, covering most of the coding sequence were used to test the sensibility of our reaction conditions. Results: All the 90 mutations (63 substitutions and 27 insertions/deletions) were detected, including the 14 not detected by the heteroduplex technique. We subsequently screened DNA from 36 HHT unrelated patients with a confirmed clinical diagnosis. We found a mutation in 28 patients, corresponding to a mutation rate of 78%. Twenty mutations were found in ALK1, including 13 missense mutations, 4 insertions/deletions, 2 splice site mutations and 1 nonsense mutation. Nine mutations were found in ENG, including 5 insertions/deletions, 1 missense mutations, 1 nonsense mutation, and 2 splice sites mutations which were confirmed on cDNA level. Ten mutations were novel. A patient had a mutation in both genes. Segregation study is in course in his family. Conclusions: Compared to systematic sequencing, dHPLC is a more simple and faster technique, with a lower cost. It appears to have a much higher sensibility than the heteroduplex technique and then, dHPLC is a good screening method for a frequent disorder like HHT, caused by mutations in, at least, two different genes.

P0734. Screening of six candidate genes involved in the TGF- β /BMP pathways in HHT patients without mutation in ENG or ALK1

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Background: About 10% of HHT patients have no mutation in ENG or ALK1, even after extensive screening. Linkage analysis is hampered by the age-related penetrance of the disease, the wide variability of the phenotype and phenocopies. Candidate gene study may, indeed, represent a valuable alternative approach in HHT.

Methods: RNA extracted from lymphoblastoid cells was used for cDNA sequencing of six genes, SMAD1, SMAD4, SMAD5, SMAD7, BMPR1A and BMPR2, in 19 HHT patients without mutation in ENG and ALK1. RNA was extracted from cultured endothelial cells (EC) from hepatic dilated vessels of one HHT2 transplanted patient to test gene expression.

Results: A novel single-base silent substitution was found in BMPR1A. A novel SMAD1 non conservative substitution was found in one patient

(c.738G>C), changing a poorly conserved methionine to an isoleucin. Two novel conservative variants were found in SMAD7 as well as a novel non conservative substitution in one patient (c.738G>C), changing a glycine to an arginine. This latter variant was absent in controls and was not transmitted to the two affected daughters. All of these genes were expressed in EC, except BMPR1A.

Conclusions: Our results are not in favor of a major role of these genes in HHT. Mutations of SMAD4 gene are not a common cause of HHT classical disease, i.e. in absence of JP. The lack of expression of BMPR1A in EC cells is in accordance with the absence of reported features of HHT in patients JP with a mutation in this gene.

P0735. Molecular study of *WISP3* in 9 families originating from the Middle-East and presenting with progressive pseudorheumatoid dysplasia: identification of two novel mutations, and description of a founder effect

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We hereby report the molecular study of the *WISP3* gene in 9 unrelated consanguineous families originating from the Middle-East: 3 from Lebanon, 5 from Syria, and 1 from Palestinian Bedouin descent, all affected with Progressive Pseudorheumatoid Dysplasia (PPD). Five different sequence variations were identified in the *WISP3* gene, two of them being new mutations: the c.589G>C transversion at codon 197, responsible for a splicing defect (A197fsX201); and the c.536_537delGT deletion (C179fsX), both in exon 3. In all other families, the affected patients were homozygous for a previously described nonsense mutation, namely c.156C>A (C52X). Interestingly, in the latter families, the C52X mutation was always found associated with a novel c.248G>A (G83E) variation, suggesting the existence of a founder effect.

P0736. The *NSD1* transcript defective in Sotos syndrome escapes nonsense-mediated decay (NMD) in leukocytes and is alternative spliced in subsets of patients and controls

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Sotos syndrome (Sos), characterized by large head circumference, dysmorphic facial features, advanced bone age, and mental impairment, belongs to the family of overgrowth syndromes. The disorder is due to haploinsufficiency of the *NSD1* gene. In the Japanese population ~50% of Sos patients are deleted for the entire *NSD1* locus, whereas only ~10% of Caucasian patients harbour a deletion. The remaining Sos patients have point mutations or indels in *NSD1*; more than 100 different aberrations have been identified so far. The 23 exons give rise to an open reading frame of 8088bp, which makes mutation screening quite laborious and expensive. We have sequenced *NSD1* leukocyte cDNA isolated from genetically verified Sos patients and control individuals. Surprisingly, both point mutations and indels were confidently detected at the cDNA level. A subset of patients and controls exhibited a distinct splicing pattern, where the proportion of alternatively spliced transcripts varied quite extensively. Thus, although Sos is caused by haploinsufficiency of *NSD1*, the mRNA apparently escapes nonsense-mediated decay in leukocytes. This suggests the possibility of cDNA mutation screening. Moreover, the observed variation in splicing patterns could potentially explain the clinical variability observed among patients with Sotos syndrome.

P0737. Epigenetic status of the *SNRPN* gene in human spontaneous abortions

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15-20% clinically recognized pregnancies end in early embryonic death. For approximately 40% of abortions the genetic reasons for developmental abnormalities are unknown. Probably, in some cases the reproductive wastage may be due to defects of imprinted genes

essential for early embryonic development. Uniparental disomy (UPD) is the most studied form of loss of imprinting gene function in human reproduction. But literature data have shown only a minor contribution of UPD to embryonic lethality. It is possible that chromatin remodeling changes connected with abnormal DNA methylation during early embryogenesis, rather than abnormalities in chromosome segregation *per se*, may have deleterious effects on imprinted gene function. The aim of our work was to analyze the methylation patterns of the promoter region of *SNRPN* gene (15q11) in extraembryonic tissues (extraembryonic mesoderm and cytotrophoblast) of 40 first-trimester spontaneous abortions with normal karyotype and 6 induced abortions as a control group. DNA methylation of the *SNRPN* promoter on the maternal homolog only was found in all studied embryos. This result shows a normal epigenetic status of *SNRPN*. No differences in the methylation pattern of *SNRPN* between spontaneous and induced abortions were found. Probably, methylation changes of the *SNRPN* promoter make no significant contribution to the early lethality of embryos with normal karyotypes. However, further data with analysis of other imprinted genes is necessary to determine the effects of loss of imprinted genomic loci functions during early stages of human embryonic development.

P0738. RNA analysis in IPAH patients reveals mutations in *BMPR2* missed by routine DNA screening.

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Heterozygous mutations in the gene bone morphogenetic protein receptor II (*BMPR2*) underlie idiopathic pulmonary arterial hypertension (IPAH). Direct sequencing of our familial cohorts has left approximately 30% without identified mutations. We hypothesise that a proportion of our PAH cohorts have mutations which go undetected by current screening methods.

To investigate this hypothesis we performed mRNA analysis on 4 patients with mutations in *BMPR2* and 6 familial patients without mutations, all of which genomic DNA has previously been screened. Total RNA was extracted from peripheral blood samples and RT-PCR performed. We confirmed that mutation IVS7 +3del T leads to aberrant splicing of exon 7. In addition, we identified a deletion of exon 2 in FAM19, subsequently confirmed by multiplex ligation-dependent probe amplification (MLPA) using genomic DNA.

Allele specific analysis of common coding polymorphisms was carried out on 6 patients and 3 unaffected relatives using quantitative primer extension assays. FAM03 was identified as having a marked reduction of one allele. No mutation identified by RT-PCR suggesting the defect may reside in the unscreened promoter region. This case alone demonstrates the potential for allelic expression studies as a method of screening pathogenic defects. The assay also confirmed a reduction of the mutant bearing allele in two cases with previously identified mutations.

These results indicate that a proportion of pathogenic defects are not detected by current DNA screening methods. RNA analysis can form a valuable part of mutation screening strategies to identify *BMPR2* mutations in PAH, and also improve understanding of their functional consequence.

P0739. Spectrum of *GJB2* mutations in autosomal recessive non-syndromic deaf patients of Kashan, Iran

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Hearing loss is the most common sensory defect in humans, affecting 1 in 1000 neonates, with over half of these cases predicted to be hereditary in nature. Most hereditary hearing loss is inherited in a recessive fashion, accounting for approximately 80% of non-syndromic hearing loss (NSHL). Mutations in *GJB2*, which encodes connexin 26 subunit beta 2, are a major cause of inherited deafness in most populations. We studied 35 probands with ARNSHL from 35 families living in the historic city of Kashan (population 40,000), in Esfahan

province in central Iran. Mutation screening of GJB2 was performed by amplification refractory mutation system (ARMS)-PCR for the detection of 35delG. We then analyzed all samples, excluding 35delG homozygote samples, by DHPLC and direct sequencing. In this study, we identified three patients homozygous for 35delG, one homozygous for the 312del14 mutation and two patients with the polymorphism V153I. Based on our data the 35delG was the most prevalent mutation in this population.

P0740. VLA-4 and Multiple Sclerosis in Italian population.

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In Multiple Sclerosis (MS), an autoimmune disease of the central nervous system caused by an interplay of environmental and genetic factors, recurrent infiltration of lymphocytes and monocytes producing inflammation in the brain and spinal cord, with consequent demyelination and axonal degeneration. Trafficking of these white cells into the CNS results from the binding of the cell adhesion molecule alpha4beta1-integrin (very late antigen-4 VLA-4) with its corresponding ligand, vascular cell adhesion molecule (VCAM-1), expressed on brain endothelial cells. Recently, several studies demonstrated that treatment of MS patients with an antibody against alpha-4 subunit, which inhibits the interaction of VLA-4 with VCAM-1, prevents or limits the development of brain lesions. The effects of this antagonism of alpha-4 integrin on the formation of brain lesions are so far unknown. We hypothesized that, to understand the autoimmune inflammatory response in MS, the polymorphic VLA-4 gene could be involved as candidate for genetic susceptibility or drug resistance to the disease. We investigated the association of two single nucleotide polymorphisms at position 269 in the promoter region of exon 1 and 3061 in the exon 24 of the VLA-4 gene, through a case-control study involving 280 Italian patients of the Calabria region with definite MS and 255 age-, gender- and ethnicity-matched healthy controls. Our results showed no significant differences in the distribution of the two polymorphisms between MS patients and controls, suggesting that, despite a biological plausibility, this VLA-4 gene polymorphisms are not a significant genetic risk factor for the susceptibility to MS in Italian patients.

P0741. Fas-dependent apoptosis at interaction of tumor cells and lymphocytes

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Prognosis of tumor growth depends on the capacity of tumor cells for induction of apoptosis in immune system cells and on the capacity of immune system cells to induce apoptosis in tumor cells. The aim of this study was to reveal expression of Fas and FasL in mouse hepatoma MH-22a, histiocytic sarcoma J-774 and syngenic splenocytes on combined cultivation in vitro by indirect immunofluorescence staining. The expression of Fas in cultures of MH-22a, J-774 and in clonal lines after their combined cultivation in vitro with syngenic splenocytes fluctuated from 5 to 80 in hepatocytes and from 10 to 100 in histiocytes. In splenocytes the expression of Fas rose to 100 %. Expression of FasL in hepatocytes was not seen and it rose to 70 % in histiocytes. Expression of FasL in splenocytes was about 40-70 %. Expression of Fas and FasL was not seen in control cells in experiments with hepatocytes and splenocytes. Expression of Fas in experiments with clonal lines J-774 and splenocytes was seen in only half of cases, as in histiocytes and splenocytes. In controls, expression of Fas in histiocytes and splenocytes was revealed in half of cases and expression of FasL in histiocytes was from 60 to 100 % and in splenocytes from 0 to 50 %. These experimental results on expression of Fas and FasL in tumor cells of different histogenesis at their interaction with syngenic splenocytes has shown that Fas-dependent apoptosis could allow tumor cells to escape from the immune system of the organism.

P0742. SYT-SSX fusion genes and prognosis in synovial sarcoma: a retrospective study of 51 patients from two institutions

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Synovial sarcoma (SS) is characterized by the t(X;18)(p11;q11) chromosome translocation, which results in generating either SYT-SSX1, SYT-SSX2 or, infrequently, SYT-SSX4 fusion gene. The association of SYT-SSX fusion types with morphology and prognosis in SS has been confirmed in several studies. In the present study we characterized SYT-SSX fusion types and evaluated the impact of two different fusions on clinical course of 51 patients with primary SS. The SYT-SSX fusion transcripts were detected in 47/50 (94%) of tumors with adequate RNA for RT-PCR analysis. Contrary to our expectations and some other studies, we could not confirm strong association of the fusion type and histology observed previously. Furthermore, we could not confirm the impact of the fusion type or any clinico-pathological variable on survival of patients with synovial sarcoma. Patients whose tumors had the SYT-SSX1 fusion showed a trend toward better survival, although the difference between survival curves was not significant.

Slovenian group of patients is interesting with respect to several molecular findings. Thus, tumors with SYT-SSX2 fusion predominated in this group (73.5%), contrary to Dutch group, where tumors with SYT-SSX1 fusion predominated (61.5%). Furthermore, we found unusually high proportion of biphasic tumors with SYT-SSX2 fusion in Slovenian group (78%).

In conclusion, neither the association between genotype and morphology of SS, nor prognostic value of SYT-SSX fusion type is absolute as it appeared in early studies.

P0743. Molecular basis of holoprosencephaly: large deletions account for at least 10% in conceptuses and 1.5% in live births

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Holoprosencephaly (HPE) is a common developmental defect affecting the forebrain and the face. The disease is genetically heterogeneous. We have collected 650 samples, 300 of them corresponding to typical HPE. We have systematically analysed the four main genes of HPE (SHH, ZIC2, SIX3 and TGIF), and identified 46 point mutations. Studying of two additional genes, GLI2 and TWSG, is in progress, and we have found 3 missense mutations (two in GLI2 and one in TWSG) as yet. Point mutations, therefore, have been identified in only 17% of the typical HPE cases (20.5% in living children and 12.5% in fetus). These data suggest either other alterations in the known HPE genes like large deletions, or the implication of other genes not yet identified.

In order to detect microdeletions in the four main HPE genes, we used real time quantitative PCR. 10% of conceptuses and 1.5% of born alive children presented deletions. So this kind of alteration is significant and must be tested as a matter of routine for each sample analysed for point mutations. That's why we developed the QMPSF method which is a low cost method and allows the screening of several genes in the same experiment.

Moreover we initiated a pangenomic approach by CGH array. Genes residing in these potential deleted regions will be considered as candidate genes for HPE.

P0744. A novel missense mutation in a C2 domain of OTOF results in autosomal recessive sensorineural hearing loss

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Screening of 12 Turkish families with autosomal recessive nonsyndromic sensorineural hearing loss without GJB2 and mtDNA A1555G mutations for 11 previously known loci revealed a family in which the phenotype segregated with the DFNB9 (OTOF) locus. Three affected children were later found to carry the novel homozygous c.3033T>C (p.Leu1011Pro) mutation in exon 25 of OTOF. Both parents

were heterozygote for the mutation. p.Leu1011Pro alters a conserved leucine residue in the C2D domain of otoferlin and is negative in 100 chromosomes obtained from normal hearing Turkish persons. This mutation expands the mutational spectrum in *OTOF* and points out the functional importance of C2 domains in otoferlin.

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P0745. Cohen syndrome: Mutational and transcriptional analysis of *COH1*

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Cohen syndrome is a rare autosomal recessive disorder, clinically highly variable and mainly characterized by developmental retardation, craniofacial dysmorphism, retinal dystrophy, and neutropenia. In 2003, a novel gene, *COH1*, on chromosome 8q22 was described, and we and others have identified mutations in patients with Cohen syndrome therein. Here we describe new molecular findings in thirteen patients with Cohen syndrome, descending from eight families originating from France, Germany, Poland, Turkey, and the U.K., with mutations in *COH1*. We identified fourteen different mutations, nine of these were novel, including four nonsense mutations, four frame shift mutations, and one potential splice site mutations. Our data contribute to further confining the phenotypic spectrum of Cohen syndrome; a consistent genotype/phenotype correlation, however, has not been established so far. All data released until now indicate that Cohen syndrome is mainly caused by mutations in *COH1* that result in a defective *COH1* protein through frame shift or nonsense sequence alterations. The lack of a second pathogenic mutation in some patients points to the existence of further alternative exons and/or other transcripts of *COH1*. Therefore, we have embarked on a detailed analysis of *COH1* transcript variants by RT-PCR and Northern hybridization. Furthermore, we are studying the expression of *COH1* in humans and mice with respect to the different splice forms. Localization and specificity of the protein, which is similar to VPS13p from yeast and therefore supposed to be involved in intracellular protein sorting, are being characterized in order to shed light on the molecular pathology of Cohen syndrome.

P0746. Biochemical and molecular diagnostics of peroxisomal disorders in Slovakia from 1995 to 2004.

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Introduction: The group of peroxisomal disorders now includes 17 different mostly severe diseases. Although different classifications have been proposed through the years, there is growing consensus to use a classification in which two groups of disorders are distinguished: (a) the disorders of peroxisome biogenesis and (b) the single peroxisomal enzyme deficiencies. Except of X-linked adrenoleukodystrophy (X-ALD), all diseases are based on autosomal recessive type of inheritance.

Methods: In peroxisomal diseases with defect in β-oxidation pathway determination of very long chain fatty acids (VLCFA), phytanic acid,

pristanic acid, pipecolic acid and plasmalogens were performed by Gas Chromatography/Mass Spectrometry (GC/MS). In some cases digitonin permeabilisation of cultured fibroblast was realised. Molecular diagnostic involved sequencing of chosen exons of *PEX1* and cDNA sequencing was used to detect mutations in *ABCD1* gene causing X-ALD. Primary hyperoxaluria and mevalonic aciduria were determined by GC/MS analysis of urinary organic acids.

Results: Two patients with peroxisome biogenesis disorder and three cases of nongeneralised peroxisomal disease with fatal course were revealed. X-linked adrenoleukodystrophy with variable phenotypes - childhood cerebral, adrenoneuromyeloneuropathy, adult cerebral and until now asymptomatic form (after bone marrow transplantation) have been found in 7 patients of male gender. One case of primary hyperoxaluria type I and mevalonic aciduria with hyper IgD were also diagnosed.

Conclusions: During last 10 years inherited disorders of peroxisomal compartment have been definitively proved for 14 patients. Besides mutations presented previously we present a novel mutation of *ABCD1* gene - c.971G>C (R324P).

P0747. Detection His1069Gln mutation by ARMS test and a seminested PCR in Slovak patients with Wilson disease

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Wilson disease (WD) is an autosomal recessive disorder of copper metabolism. More than 250 disease-causing mutations have been identified in *ATP7B* gene. Some of these mutations are frequent in specific populations, which may help to introduce rapid diagnostic procedures based on direct DNA analysis into routine clinical practice. The His1069Gln mutation in exon 14 is the most frequent one, accounting for approximately 30-63% of all mutations in Caucasian patients. The aim of the present work was to introduce direct DNA-based analysis into routine molecular screening for the above mutation in Slovak WD patients and to clarify its frequency in patients as well as in a control DNA samples. Two different DNA-based methods to detect His1069Gln mutation have been used: ACRS (amplification created restriction site) for Alw211 in combination with nested PCR and amplification refractory mutation system (ARMS). The reliability and discriminating power of these techniques was tested on samples of WD patients homozygous and heterozygous for His1069Gln mutation and on random DNA samples, and confirmed by direct sequencing. ARMS test seems to be more reproducible and precise. In summary, the mutation His1069Gln is the most frequent in either two copies or one copy in Slovak WD patients. In the control DNA samples it was present on 2 of 146 chromosomes. Our results represent a starting point of direct DNA diagnosis in the majority of WD patients in Slovakia. We hope, that data presented in this work might contribute to the geographical map of occurrence of His1069Gln mutation in Central Europe.

P0748. Screening of ARX in 414 families with X-linked mental retardation and report of the first maternal somatic mosaicism for the ARX gene

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Mutations in the human *ARX* gene have been seen to cause nonsyndromic mental retardation (MRX) as well as syndromic forms such as X-linked lissencephaly with abnormal genitalia (XLAG), Partington syndrome (PRTS) and X-linked infantile spasms (ISSX). The most common causative mutation, a duplication of 24 bp, was

found in families with a variety of phenotypes, but not in the more severe phenotype. The aim of the study was to access the prevalence of the *ARX* mutations in 414 families with established or probable X-linked MR (XLMR) collected by the European XLMR Consortium. We screened for mutations all the coding region of *ARX* by dHPLC and we identified 17 mutations (11 c.428_451dup, 2 insertions and 4 missense mutations (including the novel p.Pro38Ser)): 12 with MRX, 3 with PRTS and 2 with ISSX. All the missense mutations have been identified in MRX. This study confirms that the prevalence of *ARX* mutations is high in XLMR, especially in established XLMR (9%) and that the screening of *ARX* in MRX should not be limited to the duplication. Moreover, we have identified for the first time somatic mosaicism for the duplication found in an asymptomatic female, mother of two boys affected with MRX. Using a semiquantitative fluorescent PCR, we found that only 1.3 % lymphocytes in the mother harboured the duplication. Our finding is particularly important for genetic counseling and should lead to reconsider the molecular screening in sporadic cases of MR to look for mosaicism associated with the phenotypes.

P0749. Spectrum, frequencies and haplotype analysis of *GJB2* gene mutations in Slovak NSHL patients of Caucasian and Romany (Gypsy) origin

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Non-syndromic hearing loss (NSHL) is one of the most common hereditary impairment. Mutations in *GJB2* gene encoding connexin 26 - gap junction protein are causative in almost 60 % of patients. The prevailing mutation can be identified for different population, e.g. 35delG for Caucasians, 167delT for Ashkenazi Jews, 235delC for Orientals. We studied two subpopulations which are present in Slovakia - Slovak non - Romany (Caucasian origin) and Slovak Romany (Indian origin) population.

Biallelic *GJB2* gene mutations were found in 50.7 % and 28.3 % for non-Romany and Romany patients, respectively. In non-Romany subgroup, *GJB2* gene mutations 35delG, 167delT, R75W, 310del14 and 333delAA were identified. Polymorphisms V27I, M34T and E114G were also present in this subgroup. The prevailing mutation 35delG accounts for 92.2% chromosomes with NSHL causative mutation in *GJB2* gene. In Romany subgroup mutations W24X, 35delG, V37I and L90P, and polymorphisms R127H and V153I were detected. The prevailing mutation W24X accounts for 63.2 % of NSHL causative mutations in *GJB2* gene.

As in other populations of European origin the mutation 35delG was the most prevalent in Slovak non-Romany patients, while in Slovak Romany patients the most prevalent mutation was W24X, which is in consistency with high frequency of this mutation in India. In Slovak non-Romany patients testing for only 35delG mutation could reveal more than 90 % of *GJB2* mutated alleles, while in Slovak Romany patients the testing for two mutations - W24X and 35delG (second most prevalent) reveal slightly lower proportion - 80 %, of *GJB2* mutated alleles.

P0750. Molecular genetic diagnosis of Limb-girdle muscular dystrophy type 2A (LGMD2A, Calpainopathy) in Bulgaria

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Limb-girdle muscular dystrophy type 2A (LGMD2A; Calpainopathy) is caused by mutations in the muscle specific calcium-activated neutral protease 3 (CAPN3) gene, encoding the protein calpain 3.

So far, we have registered 44 families (46 patients) with autosomal recessive limb-girdle muscular dystrophy (AR-LGMD). All patients were screened for mutations in the CAPN3 gene. The diagnosis LGMD2A was genetically confirmed in 17 families (19 patients), so calpainopathy represents about 40% of all AR-LGMD cases.

In 12 LGMD2A families (13 patients) (70%) the disease was caused by the 550delA mutation in homozygous or heterozygous state.

The rest mutations in our patients are private and scattered along the whole gene sequence: Arg49His located in the muscle specific

unique sequence NS in Domain I of the protein; Arg169Gly in Domain I; Glu323X in IS1 muscle specific unique sequence in Domain II; Gly333Asp in Domain II; 1811_1812delTC in IS2 muscle specific unique sequence in Domain III; Asp753Asn in the third EF-hand of the Ca^{2+} -binding domain. Big deletions covering the whole exon 4 were suspected in two families. In 4 families the second mutation was not found.

Seven of the LDMD2A cases occurred missdiagnosed DMD/BMD and SMA type III patients. These genetically different disorders have some common clinical findings, which sometimes confuse the proper assessment of the clinical diagnose. Therefore, in genetically not confirmed DMD/BMD and SMA III cases, LGMD2A could be also considered as a possible diagnosis.

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P0751. A novel VDR gene mutation identified in a patient of Libyan descend with hereditary 1,25-dihydroxyvitamin D₃-resistant rickets

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Hereditary 1,25-dihydroxyvitamin D₃-resistant rickets (HVDRR), also known as vitamin D-dependent rickets type II (MIM: 277440) is a rare autosomal recessive disorder due to impaired responsiveness of target organs to the active form of vitamin D [1,25-(OH)₂D₃]. The disease is caused by heterogeneous mutations in the vitamin D receptor (VDR) gene, located on chromosome 12q12-q22 and encoding a member of the steroid-thyroid-retinoid gene superfamily of nuclear transcription factors. It is characterized by early onset rickets, hypocalcemia and secondary hyperparathyroidism. In addition, affected subjects exhibit elevated serum level of 1,25(OH)₂D₃, and, in most cases, total alopecia.

Several alterations in the human VDR gene have been detected, including nonsense, missense, splicing mutations, a partial gene deletion and an insertion/substitution. They can cause premature termination of the VDR protein, affect its ability to interact with DNA elements or impair the binding with vitamin D, resulting in partial or total hormone unresponsiveness.

Here we report on the clinical features and molecular basis of HVDRR in a patient originating from Libya, born from consanguineous parents, in whom we identified a novel homozygous mutation in the helix H1 of the VDR ligand binding domain.

The mutation, occurring in VDR exon 4 changes a codon for Leucine at amino acid 135 to Proline (p.Leu135Pro), involves an amino acid residue highly conserved in all vertebrates and is likely to cause HVDRR altering the correct folding of the VDR ligand binding structure.

P0752. Homozygous p.M172K mutation of the TFR2 gene in an Italian family with hereditary hemochromatosis type 3

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Hereditary hemochromatosis (HH), previously regarded as a single gene disorder, has been recognised to be clinically and genetically heterogeneous.

Five distinct genes, encoding proteins with different functions in iron metabolism, have been characterized. HFE is responsible for classical or type 1 HH (MIM# 235200); HJV and HAMP cause respectively type 2A and 2B HH (MIM# 602390 and 606464); TFR2 is associated with type 3 HH (MIM# 604250). Eventually, mutations in the ferroportin1 gene are at the base of an autosomal dominant atypical form of HH (Type 4 HH, MIM# 606069).

TFR2 mutations seem to account to relatively few cases of primitive iron overload. Only six causative TFR2 mutations in a total of about 11 Italian, Japanese, Portuguese or French families have been in fact described.

We report on two sibling of central Italy descent, in whom we identified in homozygosity the TFR2 p.M172K mutation.

The patients exhibited a phenotype characterized by severe iron overload and onset of hepatic damage at an earlier age compared to

type 1 HH.

This is the second family in whom the TFR2 p.M172K has been detected and the patients' clinical features confirm that HH type 3 is associated to a more severe phenotype than in HFE related HH.

Our findings may indicate that rarity of HH type 3 can be due to lack of systemic investigations of the whole TFR2 gene and that this gene could be responsible for the iron overload in a more significant percentage of patients, at least in some geographical areas.

P0753. The value of genetic diagnosis in hemophilia families from developing countries

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Introduction. The genetic diagnosis of hemophilia enables the early fetal diagnosis, with a possible decrease of the disease incidence. The primary prophylaxis of hemophilia, an expensive disease, would be beneficial.

Objectives. Characterization of the genetic abnormalities in hemophilia families, elaboration of a mutation database, starting point for the carriers detection and the fetal diagnosis.

Material and methods. The study involved families from Hemophilia Center Timisoara (CHT); 33 patients with hemophilia A (HA), and 14 of their mothers, and respectively, 6 patients with hemophilia B (HB) and one of their mothers were investigated. The genetic analyses were performed at "Institut für Humangenetik" Greifswald (Germany): direct genetic analysis (presence of inversions for HA, identification of the mutation type for HB); indirect analysis (study of the restriction fragment length polymorphisms - RFLPs).

Results and discussions. The presence of factor VIII gene inversion was analysed in 29 HA families; 18/26 (69.23%) of the patients with severe HA were positive (12/18 - type I, and 6/18 - type II inversions), as were all of their analysed mothers. A similar frequency of inversion was found, for the familial (57.89%) and sporadic (42.11%) forms of hemophilia. The RFLPs were analysed for 15 HA families, and one HB family; the limits of this method are underlined. Two patients with severe HB have missense mutations: codon -4 CGG (Arg)→TGG(Trp); codon 386 GGT(Gly)→GAT(Asp).

Conclusions. The mutation was identified for half of the hemophilia families, making carrier detection and fetal diagnosis possible anytime in the future.

P0754. Genetic predispose and mineral disorders in risk group of osteoporosis in North-West of Russia

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Osteoporosis is a multifactor disease with a strong genetic component. The aim of this work was to investigate distribution of DNA polymorphisms associated with osteoporosis, regarding to mineral metabolism and hormone status of patients. Cohort1 (89 patients) includes patients having severe osteoporosis with compression fractures. Cohort2 (49 patients) includes immediate relatives of the patients and patients themselves. The Taql, Pctl, and Apal polymorphisms of VDR gene and Msp20I polymorphism of the COLIA1 gene Sp1 site were studied by the PCR-RFLP method. The frequency of the osteoporosis associated PPAAtt genotype in cohort2 (28.6%) is noticeably higher than in cohort1 (21.3%). Five cases of familiar VDR gene polymorphisms were found. The frequency of the mutant homozygous genotype (ss) of COLIA1 is twice higher in cohort2 (12.5% and 6.8%, respectively). 61% of VDR and COLIA1 alleles have markers of osteoporosis. A correlation between the osteoporosis severity and polymorphisms in both genes was observed. DNAs of 69 patients were examined to determine length of AT-rich minisatellite repeats in 3'-UTR of the IL-6 gene. Individuals with prevailing homozygous genotype FF (51%) have a higher bone mass density compared to those having the CF (26%) genotype. 75% of the tested relatives have disorders of mineral homeostasis and 68.4% of them have a low serum Mg level. We have established familiar cases of reduced vitamin D content in the blood serum and enhanced concentration of parathyroid hormone. The data indicate that mineral disorders (especially Mg-related) and genetic predispose must be considered together for early recognition of osteoporosis.

P0755. Spectrum of GJB2 gene mutations in Korean deaf patients and the functional study of syndromic GJB2 mutant alleles

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GJB2 is one of the most frequent causative genes in congenital deafness. It is inherited by autosomal recessive manner with some exceptions. It is known that the 35delG mutation is the most frequent type of mutation in white populations, and 167delT among Ashkenazi Jews and R143W in Africans. In this study, we investigated the profile of GJB2 mutation in 93 Korean deaf patients. GJB2 nucleotide sequence analysis revealed 7 different mutations including 2 novel mutations (R75L and R143W). Fourteen percentile of total objectives has two or one GJB2 mutation. The most frequent allele was 235delC, which was found in four homozygous patients and two compound heterozygous patients. We also characterized two kinds of dominantly inherited GJB2 alleles with skin abnormalities. To functional study of these two alleles, we transfected normal or the mutant type of GJB2 genes into the gap junctional intercellular communication (GJIC) deficient HeLa cells. When we examined the GJIC with scrape-loading transfer of Lucifer yellow, the normal transfected showed the active whereas mutant or vector only control transfected did not show any sign of GJIC. The change of GJB2 subcellular localization indicates that the failure of membrane targeting or plaque formation result in the impaired GJIC.

P0756. Allele dosage dependent penetrance of RET protooncogene in Israeli Arab inbred families segregating Hirschsprung disease.

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Hirschsprung disease (HSCR) is characterized by intestinal obstruction resulting from the absence of ganglion cells in a portion of the intestinal tract. The main mutations identified in the major gene, *RET*, associated with isolated HSCR, appear to be dominant loss-of-function mutations with incomplete penetrance and variable expressivity. We have ascertained two interrelated consanguineous Israeli Arab families. In one family there were 3 female siblings affected with total colonic aganglionosis; in the second family, there was one male affected with short-segment Hirschsprung disease (S-HSCR). Linkage to the *RET* locus was found using polymorphic genetic markers. Sequencing of the *RET* gene showed the heterozygous splicing mutation (IVS6+5 G>A) in a patient with S-HSCR. This mutation impairs the consensus donor splice site. Accordingly, only 1/8 heterozygous individual harbours the S-HSCR phenotype. Interestingly the IVS6+5 G>A mutation was found at the homozygous state in 2 females affected with total colonic aganglionosis, suggesting a gene dosage effect on the penetrance and expressivity of the HSCR phenotype. Homozygous *RET* gene mutations causing HSCR are extremely rare. While the heterozygous IVS6+5 G>A is of low penetrance (12.5%) for S-HSCR disease, there is a full penetrance for the homozygous state for total aganglionosis. As suggested in other inbred populations segregating weakly penetrant *RET* predisposing allele (Mennonites), our data support that the penetrance of *RET* gene mutations for the HSCR phenotype depend on: *i*) the nature of the mutation, *ii*) the allele dosage, and *iii*) the gender of affected individual.

P0757. C282Y, H63D and S65C mutations of the hemochromatosis gene (HFE) in patients with alcoholic cirrhosis

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In the present study we have investigated the HFE gene mutations and its role in the predisposition of developing alcoholic cirrhosis in Slovenian

and Croatian patients. The groups of patients have been genotyped - 147 patients with alcoholic cirrhosis, 66 alcoholics without cirrhosis and 350 healthy controls for C282Y, H63D and S65C. The analysis of these mutations were performed using polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) method. According to our results C282Y, H63D and S65C allele frequencies were 3.4%, 18.0% and 1.4% in patients with alcoholic cirrhosis, 3.0%, 12.9% and 1.5% in alcoholics without cirrhosis and 3.4%, 13.6% and 1.1% in healthy controls, respectively. Our results indicated no significant difference in the HFE allele and genotype frequencies between investigated groups, except the higher frequency of H63D heterozygotes observed in patients with cirrhosis (32.0%) than in healthy controls (19.1%) ($p < 0.05$). These results suggested that C282Y and S65C mutations were not associated to the risk of developing alcoholic cirrhosis in our populations, but the role of H63D mutation has to be elucidate in further study in a larger series of patients.

P0758. Deletion of hypotonin, a novel serine oligopeptidase, in patients with the hypotonia-cystinuria syndrome

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Hypotonia-Cystinuria Syndrome is a novel recessive disorder identified in 12 patients. They present with generalised hypotonia and failure-to-thrive, which gradually diminishes after infancy. Growth is retarded due to a growth hormone hyposecretion. All patients present with cystinuria type I within their first decade.

Isolated cystinuria type I is caused by mutations in *SLC3A1*. Screening this locus with quantitative PCR techniques resulted in the detection of deletions in all patients. In total, five different deletions ranging from 24 to 75 kb were identified. Interestingly, these deletions disrupt the coding region of only two genes, *SLC3A1* and *KIAA0436*. As *SLC3A1* causes isolated cystinuria type I, *KIAA0436* is responsible for the extended phenotype. The *KIAA0436* gene product, hypotonin, shows homology with two serine oligopeptidases (POP and OpdB) and secondary structure predictions reveal striking similarities. Moreover, amino acids forming the catalytic triad are conserved. Reactivity against FP-biotin, a biotinylated form of the serine hydrolase inhibitor DFP, was observed, indicating an active conformation. Site-directed mutation of the catalytic triad amino acids and generation of an inhibitor profile suggest that hypotonin is a novel serine (oligo)peptidase with unique catalytic properties.

P0759. Genetic analysis in Spanish cases of dominant cerebellar ataxia

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Autosomal dominant cerebellar ataxias (ADCA) are a clinically heterogeneous group of neurodegenerative disorders caused by unstable trinucleotide repeat expansions. Seven spinocerebellar ataxia genes: *SCA1-3*, *SCA6-7*, *SCA12* and *SCA17* have been cloned with the finding of an expansion of 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 222 unrelated familial and 522 sporadic and idiopathic Spanish cases of spinocerebellar ataxia. For ADCA familial cases 40.21% were *SCA3*, 28.87% *SCA2*, 11.34% *SCA8*, 7.22% *SCA1*, 5.15% *SCA6* and 1.03% *SCA17*. None of ADCA mutated cases also have the *SCA8* expansions in their reported pathogenetic range, thus we have not found coexistence of *SCA8* expansions with other SCA expansions in our SCA series. About 56% of familial ADCA cases remained genetically unclassified. No SCA mutations were detected in the 522 isolated and idiopathic cases of spinocerebellar ataxia.

P0760. Inherited disorders of bilirubin glucuronidation in Slovak population

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Three forms of bilirubin UDP-glucuronyltransferase (B-UGT) deficiency are known in humans. Absent B-UGT activity results in severe, and very rare unconjugated hyperbilirubinemia, Crigler-Najjar syndrome type I (CN-I), characterized by potentially lethal hyperbilirubinemia. Strongly reduced B-UGT activity resulting in Crigler-Najjar syndrome type II, characterized by intermediate levels of hyperbilirubinemia. Moderately reduced B-UGT activity causing the mildest form of unconjugated hyperbilirubinemia Gilbert syndrome (GS), one of the most common inherited disorders, with prevalence of approximately 10-13% in Caucasians. All three diseases are caused by mutations in UDP-glucuronyltransferase gene (*UGT1A1*) encoding B-UGT, which is involved in detoxification of bilirubin by conjugation with glucuronic acid. Here we report the results of molecular-genetic study of inherited unconjugated hyperbilirubinemias in Slovak population. Forty-seven families with suspected GS, a family with 4 children affected with CN-I, and a patient with CN-I were studied. Sequencing of promoter area and of all five exons of the *UGT1A1* gene in CN-I patients was performed with PCR primers designed by us. In families with suspected GS, the A(TA)_nTAA motif in the promoter area of the *UGT1A1* gene was analyzed by PCR followed by separation of the amplified products on 15% nondenaturating polyacrylamide gel. Out of 52 suspected GS patients, 49 had the most frequent GS mutation observed in Caucasians A(TA)_nTAA, 2 were heterozygous and 1 was homozygous for normal allele A(TA)_nTAA. The frequency of the A(TA)_nTAA allele was 0.96% in suspected GS patients to compare with 0.35% in 120 healthy control subjects.

P0761. Development of direct and indirect DNA-diagnostics of neurofibromatosis type 1 in Russian Federation

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder affecting ~ 1 in 3500 individuals. The disease is caused by mutations in the tumor suppressor gene *NF1*, whose mutation rate estimated to be 10-fold higher than in most other genes. We have developed the protocol for direct and indirect NF1 DNA-diagnostics. DNA samples from 35 unrelated patients (16 familial and 19 sporadic cases) were screened for the presence of mutations by SSCP, HD and microsatellite analysis; all mobility shifts were sequenced. Twelve mutations have been found and ten of them were described for the first time. Eight mutations were detected for the sporadic patients and four in familial cases. The complete spectrum of mutations has been found: small deletions and small insertions (192-195dupTGTT, 4973-4978delTCTATA, 5459delA, 998-999insA, 2415-2416dupTG, 1075delA and 2345delC), missense mutations (5498 T > A), nonsense mutations (1094C>G), splice site mutations (IVS2+1delGTGA and IVS10c+1G > A) and large deletions (deletion on markers TAGA/TAGGint27a and GTint38). For indirect DNA-diagnostics we have developed a panel of 4 intragenic microsatellite markers - TCCAint1, D17S1849, TAGA/TAGGint27a and GTint38, which were very helpful for prenatal diagnostics and for searching of long rearrangements of the *NF1* gene in families.

P0762. Improvement in identification of haemophilia carriers' by screening for gene rearrangements

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Haemophilia is a frequent X-linked bleeding disorder caused by factor 8 (F8) or factor 9 (F9) gene defects. Identification of disease-causing mutations usually relies on extensive analysis of the coding sequence

in index-cases and subsequently allows carrier status determination in at-risk women. However, in case of large deletions, which account for 5% of disease alleles, direct molecular diagnosis of carriers becomes difficult by conventional PCR. Quantitative real-time PCR can be efficiently applied once a particular deletion has been identified in an index case. Screening for gene rearrangements by semi-quantitative fluorescent multiplex PCR, recently developed to identify such heterozygous rearrangements in various diseases, represents an attractive option. We developed an assay encompassing the eight exons, the promoter region and the polyadenylation signal sequence of the *F9* gene, involved in haemophilia B. It was successfully applied to establish the carrier status of women in 5 families where various large deletions were previously found in patients. Furthermore, it identified a deletion of exons 7-8 in a woman having a low FIXc activity of 30% but no family history of haemophilia, and whose extensive screening for mutations was negative. Compared to gene dosage by real-time PCR, the method simultaneously analyses multiple gene loci and is cost effective. In addition, it is sensitive enough to detect micro-deletions/insertions, thus being a powerful diagnostic tool. Such an assay would also be advantageous as a first step screening in the diagnosis of haemophilia A, with regards to sequential analysis of the 26 *F8* gene exons.

P0763. Evaluation of CFTR gene mutation testing methods in laboratories participating in the Italian External Quality Assessment

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The Italian External Quality Assessment (IEQA) in molecular genetic testing is coordinated by the Istituto Superiore di Sanità (ISS) and supported by the Italian Ministry of Health.

IEQA organization is described in Taruscio et al. ⁽¹⁾. Gene test for cystic fibrosis (CF) is the most frequently performed in Italian laboratories (more than 30000 tests requested in 2002) ⁽²⁾.

We present an overview of results and impact of the IEQA in CF; moreover, Italian data are discussed in the European context.

In particular the evaluation of CFTR gene mutation testing methods will be discussed.

At National level:

1. Oligonucleotide Ligation Assay PCR (OLA-PCR) and Reverse Dot Blot (RDB) analysis were the most frequently used testing method
2. The majority of Italian participating laboratories made use of commercial kit (i.e.: INNO LiPa CFTR12 and 17 + Tn Innogenetics; OLA CF assay - PE, etc.).

We'll focus on methods used by laboratories and frequent errors.

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1.Taruscio D. et al., *Clin Chem Lab Med* 2004; 42: 915-21

2.Dallapiccola et al., *Analysis* 2/3. 2004

P0764. Autosomal Dominant Cerebellar Ataxia mutations in 104 Italian ataxic patients.

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Autosomal Dominant Cerebellar Ataxias (ADCAs) are a heterogeneous group of neurodegenerative disorders for which at least 25 SCA (Spino-cerebellar Ataxia) loci are known. In eleven of these loci the gene was identified and the mutation found to be an expansion of a microsatellite sequence in coding or non-coding regions. Two ADCAs are caused by point mutations in FGF14 (Fibroblast Growth Factor 14) and in PRKCG (Protein Kinase C γ) genes. CACNA1A gene mutations, responsible for Episodic Ataxia type 2, have sometimes been associated with an AD permanent progressive ataxia phenotype. SCA was also associated with premutation of FragileX CCG repeat. The relative frequency of the various genetic types of ADCAs appears to be remarkably different between ethnic groups. In order to contribute to the assessment of SCAs relative frequency in Italian population, 104 Italian ataxic patients were collected and screened for mutations of

known SCA genes. The screening while confirming the high frequency of SCA1 and 2 and the low frequency of SCA3 in Italy as compared to other countries, showed: a) a low frequency of SCA 6,7,8,17; b) presence of SCA14 and EA2 point mutations; c) presence of atypical Friedreich Ataxia cases with pseudodominant inheritance; d) absence of SCA10-12 and Fragile X permutation. Molecular diagnosis could be assigned to 70% of familial and only in 5% of sporadic cases. The data are relevant both in a genetic epidemiology context and in devising priorities for molecular tests in Italian ataxic patients.

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P0765. Positional cloning of four candidate genes for autism: a possible role for neuron vesicle trafficking in the pathogenesis of autism.

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Autism is a neurodevelopmental disorder of unknown cause and pathogenesis. The identification of genes involved in autism is expected to increase our understanding of the pathogenesis of this disorder. We initiated a positional cloning strategy, starting from four unrelated persons with idiopathic, non-familial autism carrying a *de novo* chromosomal aberration.

Last year, we reported on the identification of three different genes affected by the characterized translocations: *Neurobeachin* (*NBEA*), *CLIC4* (chloride intracellular channel protein) and *amisyn*. Recently, the *C10orf74* gene was found to be affected by the paracentric inversion present in the fourth patient. This gene encodes for a predicted protein with high homology to proteins in human and several other species, most of which have not been characterized yet. The best characterized homolog is yeast *Yop1p*, which interacts with the yeast Rab GTPase *Ypt1p* required for vesicular ER-to-Golgi transport.

The involvement of these genes in the regulated secretory pathway of LDCVs is currently under study by means of RNAi-mediated gene knockdown in the *βTC3* neuroendocrine cell line. For neurobeachin and *amisyn*, we showed that disruption of gene expression results in a significant increase of regulated secretion, suggesting a role for both proteins as negative regulators of vesicle trafficking and/or fusion. Similarly, overexpression of *Yop1p* (*C10orf74* yeast ortholog) has been reported to block ER-to-Golgi membrane trafficking.

In conclusion, these data suggest a role for *NBEA*, *amisyn* and *C10orf74* as negative regulators of neuron vesicle trafficking. Moreover, it implies that vesicle trafficking in neurons may be involved in the pathogenesis of autism.

P0766. Polyalanine expansions, missense and frameshift mutations of the *PHOX2B* gene result in loss of function via different mechanisms

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Heterozygous mutations of the *PHOX2B* gene account for disorders of the autonomic nervous system either isolated or combined, including: congenital central hypoventilation syndrome (CCHS), tumours of the sympathetic nervous system (TSNS) and Hirschsprung disease. In CCHS, the prevalent mutation is an expansion of the 20 alanines stretch ranging from +5 to +13 alanines, while frameshift and missense mutations are found occasionally. Importantly, as opposed to polyalanine expansions, frameshift mutations predispose to TSNS.

To further investigate phenotype/genotype correlation, we performed both transactivation and DNA binding assays on wild type and mutant *PHOX2B* proteins. Furthermore, as already reported in polyalanine expansion diseases, nuclear and/or cytoplasmic *PHOX2B* aggregations were investigated by immunofluorescence and gel filtration in protein with expansions ranging from +5 to +13. Except for the +5 alanines

expansion, all mutations resulted in reduced PHOX2B transactivation. However, the disease causing mechanism seems to vary according to the nature of the mutation. On the one hand, *in vitro* DNA binding was altered for both homeodomain missense mutations (R100L and R141G) and expansions. On the other hand, while cytoplasmic aggregation was observed only for expansions of +9 and above, *in vitro* spontaneous formation of oligomers occurred starting at +5 alanines expansion. Finally, considering frameshift mutations, mRNA decline could be excluded in tumoral tissues.

Altogether, our data favor a loss of function mechanism irrespective of the nature of the mutation. However, as both transactivation and cellular localization were normal with the +5 alanines expansion, the disease-causing mechanism remains unclear for short alanine expansions.

P0767. Association of GABRG2 gene and polymorphic DNA-loci D5S422, D5S402 with idiopathic generalized epilepsy in Volga-Ural region of Russia.

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Idiopathic generalized epilepsy (IGE) is a common form of epilepsy accounting for 20-40% of all epilepsies. Last genetic discoveries showed that IGEs are associated with mutations in genes encoding voltage- and ligand-gated ion channels (Na^+ , K^+ , Cl^-) and some neurotransmitter receptors (GABA, dopamine).

We aimed to study association of some polymorphic DNA-loci linked to epilepsy genes with an increased risk of developing epilepsy. 125 patients from Volga-Ural Region of Russia with different forms of IGE and 150 healthy donors were enrolled in our investigation. We study polymorphic DNA-loci D5S422 and D5S402, linked to GABRG2 (gamma2 subunit of gamma-aminobutyric acid receptor) gene (5q34).

The analysis of D5S422 polymorphisms revealed some differences in distribution of allele and genotype frequencies between IGEs patients and healthy donors. The research of D5S402 polymorphisms showed that allele 2 (169 bp) is significantly frequent in patients (49, 5%) in comparison with controls (39, 7%) ($\text{OR}=1,49$; $p<0,05$). To further analyze the possible role of GABRG2 gene in epileptogenesis we determined the genotype and allele frequencies C588T polymorphism of the exon5. The allele frequencies in patient group were 0, 85 for C and 0, 14 for T.

Our results suggest that GABRG2 may be involved in epileptogenesis of common forms of IGE in Volga-Ural region.

P0768. Canavan disease in two Slovak patients

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Canavan disease (OMIM 271900) is an autosomal recessive spongy degeneration of the central nervous system and usually has fatal prognosis in 1 to 3 years. The disease is caused by deficiency of the enzyme aspartoacylase (ASPA) (EC 3.5.1.15) and characterised by increased levels of N-acetylaspartic acid (NAA), a possibly neurotoxic compound, in body fluids. The human aspartoacylase gene is located on chromosome 17p13ter and contains 6 exons, which encodes a protein of 313 amino acids.

Two unrelated patients have been diagnosed in Slovakia last year. Patient 1 a third child in family presented psychomotor retardation, hypertonic syndrome and leukodystrophy at 4 months of age. Patient 2 a first child of non-consanguineous parents presented macrocephaly, spastic quadripareisis, amaurosis, seizures and leukodystrophy at 1 year of age. The gas chromatography/mass spectrometry analyses of organic acids in urine revealed high excretion of NAA in both cases.

To elucidate the cause of disease at molecular level we analysed the ASPA gene by direct sequencing of polymerase chain reaction products of all 6 exons from genomic DNA of patient 1. We have found homozygous missense point mutation 914 C>A in exon 6 changing alanine 305 to glutamic acid (A305E), which is the most common mutation among European patients of non-Jewish origin and leads to complete loss of ASPA enzyme activity. Both parents were found heterozygous for A305E. The molecular genetic analysis of patient 2 has revealed heterozygous state for A305E mutation. The search for the second pathologic mutation is still in progress.

P0769. Genetic analysis of the two major ADHSP genes in genes in Bulgarian patients with hereditary spastic paraparesis

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INTRODUCTION: Hereditary spastic paraparesis (HSP) is a neurodegenerative disorder affecting the most distant parts of the corticospinal tracts in CNS with a main clinical characteristic, spasticity of the lower limbs. The most common genetic form of the disease among European population is the autosomal dominant (ADHSP). Mutations in two major genes - spastin and atlastin, contribute to about 50% of ADHSP cases.

MATERIALS AND METHODS: We performed a mutation screening of spastin and atlastin genes, using direct sequencing, on a cohort of 40 Bulgarian patients with different ethnic background (Bulgarian, Turkish and clinical diagnosis of HSP).

RESULTS AND DISCUSSION: We detected 4 spastin and 2 atlastin mutations. Five of them are new and one spastin mutation is already reported. All mutations in both genes are different single nucleotide substitutions. The 2 atlastin mutations and 2 of those found in the spastin gene result in amino acid changes. The other two spastin mutations affect different splice sites. The family history of the disease suggests AD or a dominant (D) inheritance for 11 out of 40 examined families. We found the genetic defects in 6 out of those 11 AD/D HSP families with clear family history for HSP in at least 2 generations. In one family with an atlastin mutation we observed incomplete penetrance of the disease. We did not find any mutation in autosomal recessive or sporadic cases. In conclusion, the analysis of the two major ADHSP-related genes identified the genetic defects in about 55% of Bulgarian ADHSP families.

P0770. Promoter region of CACNA1A gene and Episodic Ataxia Type 2

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Episodic Ataxia type 2 (EA2) is a rare autosomal dominant disorder characterised by attacks of vertigo, visual disturbance, dysarthria, ataxia and interictically by cerebellar deficit of variable severity. The disorder is due to protein truncating or missense mutations leading to a loss of function of the Cav2.1 subunit, the pore-forming subunit of voltage-gated P/Q type calcium channels, coded by CACNA1A gene. Cav2.1 has a neuron specific expression and a very high number of isoforms with different biophysical properties. Its expression regulating mechanisms should therefore be remarkably complex. So far, only the coding regions of human CACNA1A gene are known, while expression regulating machinery remains unexplored. As a first step towards the understanding of the human gene regulation we have identified a new region at 5' of CACNA1A gene, that has a neuron-specific transcriptional activity. Within this region a mutation has been identified in a patients affected with EA2 for whom mutations of the CACNA1A coding region could be excluded. The mutation, not present in 230 random chromosomes, deletes 5 nucleotides and is predicted to disrupt a putative binding site for Interferon Regulating Factor 1 or 2. In a preliminary functional analysis the mutation completely abolished the transcriptional activity of the wildtype fragment. The region under study opens the way to a) increased probabilities to detecting mutations and exploring pathogenesis of CACNA1A-related disorders; b) detecting expression modulating polymorphisms; c) identifying signalling pathways involved in the expression of P/Q calcium channels as possible targets for drug development.

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P0771. Should we screen for the Fragile-X premutation in the older population presenting with ataxia?

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In 2001, Hagerman described the occurrence of a late-onset neurological disorder in male carriers of the fragile-X premutation.

This disorder, designated the Fragile-X Tremor Ataxia Syndrome (FXTAS), consists of progressive intention tremor, cerebellar ataxia and cognitive decline. Since the first description additional symptoms were documented and diagnostic criteria proposed. More recently, the occurrence of FXTAS was also reported in female carriers of the FMR1-premutation, presenting with tremor and ataxia.

Since cerebellar ataxia is one of the cardinal features, we performed FMR-1 premutation screening in 122 male and 131 female patients, older than 50y, who were referred for testing of the spinocerebellar ataxia (1, 2, 3, 6, 7) genes and who were negative. In the group of male patients we found 5 with an *FMR-1* premutation. In four of them, a definite diagnosis of FXTAS was made, based on the clinical and radiological diagnostic criteria. In the group of 131 females, we did not detect a patient with an *FMR-1* premutation, with all repeat alleles within the range of 10-49.

Combining our results and the results of similar studies, we propose to include *FMR-1* analysis in the molecular diagnostic work-up of older ataxia patients, even though the *FMR-1* premutation seems not to be a major cause of late onset ataxia in females. *FMR-1* premutation should especially be excluded when additional FXTAS symptoms are present. Moreover, the important counseling aspects and the identification of several new fragile X patients in the families of the 5 identified FXTAS males, further justifies this screening.

P0772. Prenatal and Postnatal Molecular Study in Serbian SMA Families - two years experience

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Spinal muscular atrophy (SMA), with a prevalence of 1 in 10000 and a carrier frequency of about 1/40, is the second most frequent autosomal recessive disease. According to the age of onset and severity of the clinical manifestations, SMA is classified into three types (SMAI,II,III). Two candidate genes, SMN and NAIP, were isolated from chromosome region 5q11.2-q13.3. SMN gene exists in two nearly identical copies, telomeric and centromeric, but only deletion/mutation in SMN_t is seen to cause SMA (>98%). Deletion of NAIP gene is observed more often in severe SMA (65%), but there is no evidence that these genes play a role in the pathology of the disease.

In this report we present the molecular analysis of the SMN and NAIP gene in 26 SMA cases, over a two-year period. DNA extracted from blood samples, CVS and amniotic fluid was amplified by PCR. We amplified exons 7 and 8 of SMN gene and exons 5 and 13 of NAIP gene. Restriction enzymes digestion was used to separate SMN_t and SMN_c. Results revealed the homozygous deletions of exons 7 and 8 of the SMN gene in 84% (16/19) and deletion of exon 5 of the NAIP gene in 4 SMAI cases. Seven prenatal samples, from pregnant women with affected child, have been tested (one case was with deletions of exons 7 and 8).

These findings have important implications for genetic counseling and pre- and postnatal diagnosis of SMA.

P0773. DHPLC analysis of the *EXT1* and *EXT2* genes in patients with multiple osteochondromas.

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Hereditary multiple osteochondromas (MO) is an autosomal dominant bone disorder characterized by the presence of bony outgrowths (osteochondromas or exostoses) on the long bones. Standard mutation analysis performed by sequencing analysis of all coding exons of the *EXT1* and *EXT2* genes reveals a mutation in approximately 80% of the MO patients.

We have now optimized and validated a DHPLC based protocol for screening of all *EXT1* and *EXT2* coding exons in a set of 49 MO patients with an *EXT1* or *EXT2* mutation. Under the optimized DHPLC conditions, mutations were detected in all patients. These include 20 previously described mutations and 29 new mutations, including 20 new *EXT1* and 9 new *EXT2* mutations.

The protocol described here therefore provides a sensitive and cost sparing alternative for direct sequencing analysis of the MO causing genes.

P0774. Complex molecular-genetic analysis of patients with spinal muscular atrophy

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease, characterized by the loss of motor neurons in the spinal cord, leading to proximal, symmetrical limb, and trunk muscle weakness. The gene implicated in SMA is the survival motor neuron gene (SMN) located on chromosome 5q13. In humans, the SMN gene is duplicated with telomeric copy (SMN1) and centromeric copy (SMN2). The differences between these highly homologous genes result in alterations in their RNA expression patterns. Homozygous SMN1 deletions were found in ca 95% of SMA cases. Point mutations, micro-deletions or insertions and small duplications are scattered through all SMN1 exons in ca 5% of SMA cases. SMN2 may occur in more than two copies per diploid genome. Studies of correlation between disease severity and the number of SMN2 copies have shown an inverse relationship.

In our study we present results of SMA diagnostics using 1) PCR-restriction analysis for detection of SMN1 homozygous deletion; 2) real-time PCR for detection of SMN1 copy-number - identification of SMA carriers and SMA patients with SMN1 deletion on one chromosome and point mutation on the second one; 3) denaturing high performance liquid chromatography and sequencing for detection of point mutation in patients with one SMN1 copy; 4) real-time PCR for detection of SMN2 copy-number - determination of correlation between SMN2 copy-number and clinical findings (SMA of type I, II or III).

This work was supported by the Ministry of education, youth and sports (FRVS 2422/2005).

P0775. Mutation screening of genes associated with long QT syndrome using PCR and SSCP methods

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The congenital long QT syndrome (LQTS) is a hereditary cardiac disorder with known risks for ventricular fibrillations, syncopes, seizures and arrhythmic events potentially leading to sudden death. At least seven genes linked to LQTS were identified so far, mostly encoding various structural and regulatory subunits of cardiac membrane ion channels.

The aim of our study was to implement mutation screening based on polymerase chain reaction (PCR) and single strand conformation polymorphism (SSCP) in three genes associated with LQTS: *SCN5A* (27 coding exons divided into 34 parts, *KCNE2* (1 exon) and *KCNJ2* (1 exon divided into 6 parts). Using primers which cover whole coding regions of these genes, we optimized PCR conditions for all studied fragments and used this methodology for amplification of DNA samples of 66 patients with suspected LQTS. PCR fragments were subsequently analyzed by SSCP. Samples which have shown irregularity in pattern of bands on SSCP analysis were purified and sequenced. Total amount of 1961 samples was analyzed so far.

In exon 28 of the *SCN5A* gene we found a single-nucleotide polymorphism C5607T. In total of 65 analyzed samples, 40 samples exposed T/T genotype, 22 samples were of C/T genotype and three samples represented C/C genotype. This would indicate allele frequency of 0,785 (T allele). This observation significantly differs from results in other populations in which this SNP was previously observed (American - 0,123; Japanese - 0,46; Chinese - 0,413). No mutation directly causing LQTS was found so far.

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P0776. Genomic deletions in *STK11* in Peutz-Jeugers Syndrome detected by Multiplex Ligation-dependent Probe Amplification (MLPA)

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Peutz-Jeghers syndrome (PJS) is an inherited cancer syndrome, which results in a greatly increased risk of developing gastrointestinal polyps. Defects of the STK11 (LKB1) gene are the predominant cause of PJS. The STK11 gene has 10 exons and spans 21 Kb of chromosomal sequence on chromosome 19p13.3. Point mutations and small deletions / insertions in the STK11 gene can be detected by sequencing, DHPLC and other methods. Deletions and duplications of complete exons are usually not detected by these methods but can be detected by MLPA. A new MLPA probemix specific for STK11 was developed and covers almost all exons as well as the promoter and can be used to determine the copy number of most STK11 exons.

We analysed 36 PJS families using conventional methods for mutation detection (DHPLC or DNA sequencing) and MLPA. There were 14 families (39%) with point mutations in STK11 and 7 with genomic deletion. The nature of the deletions was quite heterogeneous.

Four cases had the entire STK11 gene deleted, one had the promoter region and exon 1 deleted, one had exons 2-9 deleted and the last one had exons 3-9 deleted. These data show that 33% of pathogenic mutations in STK11 are genomic deletions which demonstrates that the deletion in STK11 recently described by Le Meur et al. is not a rare phenomenon. We conclude that MLPA is a sensitive, robust and easy to perform technique and an important tool for deletion detection in STK11.

P0777. Integrated homogeneous genotyping assay using dried blood spots.

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Laboratories performing genetic tests need simplified methods allowing automation. Our aim was to investigate the direct use of blood disks in homogeneous PCR for genotyping of celiac disease related risk haplotypes (DQ2: HLA-DQA1*05 and HLA-DQB1*02 alleles, and DQ8: HLA-DQB1*0302 allele).

Blood samples from 134 volunteers were spotted onto 903 collection papers. A disk sample was punched and added to the reaction tube together with the PCR mix including detection probes. The reactions were sealed and moved to a thermal cycler. After cycling, the reactions were detected through the seal using time-resolved fluorometry. DELFIA Celiac Disease Hybridization Assay Kit (PerkinElmer) was used as a confirmatory test.

The homogeneous PCR assay gave detectable signals (blank+3SD) with 1 ng of input DNA. The within-run variation was below 20%. The low detection limit of the assay allowed the use of blood disk samples. When the disk sample gave detectable signal with DQA1 and DQB1 controls, the amplification was considered successful. All the blood disk samples were successfully genotyped. However, 4/134 disk samples were reanalyzed due to an unsuccessful amplification in the first analysis. Of the 134 samples, 29 (22%) and 26 (19%) had DQ2 and DQ8 haplotypes, respectively, which were in consistence with the DELFIA analysis.

The integrated homogeneous assay presents an automation-friendly and simple way for genotyping without the need for pre-processing of disk samples. The analysis of 134 dried disk samples using the integrated homogeneous PCR assay showed successful genotyping results for the celiac disease related risk alleles.

P0778. Study of the prevalence of 35delG mutation in the *Gjb2* gene among patients with ARNSHL referred to the genetic counseling center of Isfahan Welfare organization, Iran

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Hearing loss is one of the defects which has high prevalence. About 1/1000 children are born deaf and 1.5 to 2/1000 will be affected by age six.

The cause and nature of inherited deafness would become clear from identification of a known syndrome, but mostly definite diagnosis is not simple. In such cases the pattern of inheritance can only be identified through family review or drawing the pedigree. Hearing loss relates to ten genes that could be observed among the affected people who were referred to the genetic counseling center of Isfahan. We reviewed the connexin 26 gene and our results were as follows:

Among those referred to the genetic counseling center in Isfahan

about 4400 files were reviewed and hearing loss was noted. From this number 50 members of different families were contacted whose pedigree suggested autosomal recessive hearing loss. 45 persons were tested. In 30 cases connexin 26 was negative; 12 cases were positive and 3 cases are still under review. For those whose connexin 26 result was negative first class of family members were considered individually and are under more consideration. Mutations detected were 35delG 6 (13.3%); 312del14 1 (2.2%); v271/wt 1 (2.2%); Not DFNB1 2 (4.4%); Wt 1 (2.2%); 35DelG/Wt 1 (2.2%).

P0779. Quality assurance for genetic testing

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Objective: Development and testing of multi-mutation molecular quality controls. Based on evaluation of quality assurance needs for molecular testing, controls were constructed through creation of DNA constructs, assembly of material, and performance testing. Evaluation showed high quality DNA recovery by common extraction methods, expected test results by clinical test methods, sensitivity to analytical test failure conditions, and stability.

Need: Quality controls that provide multiple mutations in one sample are needed for cystic fibrosis (CF) and other genetic tests in order to monitor the entire assay, including extraction.

Construction and Assembly: DNA constructs containing 24 CFTR exons including intronic borders and carrying multiple mutations were synthesized and suspended in artificial blood matrix.

Extraction and testing: The CF Control and whole blood were extracted by common methods and amplified using CF Roche Gold 1.0. PAGE band intensities of control and genomic sample amplicons were similar. Test results were concordant with expected sequence except for known "neighboring mutation" method interferences.

Sensitivity: Sensitivity to analytical factors was demonstrated by extraction and CF Gold 1.0 testing of control in parallel with genomic samples in a simulated polymerase failure.

Stability: A CF Control was incubated at 4°C and 60°C and serially tested yielding a predicted stability of >679 days at 4°C.

Conclusions: Synthetic Controls are useful quality assurance tools.

Characteristics:

- A. Mimic genomic samples in multiplex amplification,
- B. Contain rare mutations,
- C. Allow for multiple mutation detection in one sample,
- D. Can monitor several extraction methods,
- E. Aid in setting up assays and genotype assignment.

P0780. Mutation screening in *MSX1* gene in non-syndromic cleft lip and palate patients

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MSX1 is emerging as an especially strong gene candidate in non-syndromic clefting. Support for this comes from human linkage and linkage disequilibrium studies, chromosomal deletions resulting in haploinsufficiency, a large family with a stop codon mutation that includes clefting as a phenotype, the *Msx1* phenotype in a knockout mouse. *MSX1* mutations are found in 2% of cases of clefting and should be considered for genetic counselling implications.

Aim of the study was to identify potentially etiological mutations in *MSX1* gene in CL/P and CPO patients. In this study a total of 116 independent *MSX1* chromosomes (58 unrelated patients with CL/P and CPO residing in Lithuania) were investigated.

5'UTR and exon one and intro of *MSX1* gene of all probands were screened for DNA sequence alterations by direct sequencing.

In the 5'UTR one SNP was found in 5 chromosomes (-36G>A); in the exon one one change in sequence was found - A34G (c.101C>G) in 9 chromosomes. Six different polymorphisms were found in the intron sequence.

No potentially etiological mutations in *MSX1* gene were identified.

P0781. Analysis of biotransformation system genes using 'Detoxichip'

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Most xenobiotics (carcinogens, drugs, food etc) are metabolized in the human organism by numerous enzymes of biotransformation system. Genes encoding these enzymes are characterized by significant genetic polymorphism which is thought to be connected for genetic predisposition to different types of cancer. We present a diagnostic biochip ("DetoxiChip") for genotyping of biotransformation system gene polymorphism. We used multiplex PCR followed by allele-specific hybridization on biochip for the detection of SNP. Mutations were discriminated by analyzing fluorescence intensities from individual probes on biochip. The following loci have been chosen: CYP1A1 (C4887, A4889G and T6235C), CYP2D6 (G1934A and DelA2637), GSTM1 (deletion), GSTT1 (deletion), NAT 2 (S1, S2, S3 alleles) and MTHFR (C677T).

We tested "DetoxiChip" on 30 control and more than 100 diagnostic samples. Among 715 SNPs that yielded fluorescent signals, 2 showed less than 99% of concordance, whereas 713 were performed accurately. Thus, the accuracy of the method was 99.7%. The biochip is suggested to be used for the analysis of genetic predisposition to multifactorial diseases like cancer, as well as for screening of polymorphic loci associated with individual drug sensitivity.

P0782. Rapid TPMT-genotyping for clinical applications using DNA-biochips

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Thiopurine drugs are metabolized, in part, by S-methylation catalyzed by thiopurine S-methyltransferase (TPMT). Patients with very low or undetectable TPMT activity are at high risk of severe, potentially fatal hematopoietic toxicity when they are treated with standard doses of thiopurines. Because human TPMT activity is controlled by a common genetic polymorphism, it is an excellent candidate for the clinical application of pharmacogenetics. A new molecular approach was developed to detect point mutations in the *TPMT* gene that cause the loss of TPMT activity. A fluorescently labeled amplified DNA is hybridized with oligonucleotide DNA probes immobilized on a biochip. The *TPMT* biochip can recognize six point mutations in the *TPMT* gene and seven corresponding alleles associated with TPMT deficiency. The genotyping procedure is rapid, reliable, and cost-effective and can be used for rapid screening of inactivating mutations in the *TPMT* gene. Here we present the results of biochip analysis of the 625 DNA samples in the Russian population. *TPMT* gene mutations were identified in 36 subjects. Thirty-one individual (4.96%) had genotype *1/*3A, five (0.8%) had *1/*3C, and two (0.32%) had *1/*2. The remaining 587 individuals (93.9%) had the wild-type genotype, *1/*1. The results show the feasibility of new chip-technology for pharmacogenetic testing in order to identify patients carrying the TPMT-deficiency alleles.

This work was supported by the Russian Foundation for Basic Research (grant 03-04-49355), St. Jude Children's Research Hospital International Outreach Program and by the American Lebanese Syrian Associated Charities (ALSAC).

P0783. An Evaluation of the VariantSEQR™ re-sequencing system and automated SeqScape® V2.1 mutation detection software for use in diagnostic laboratories.

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VariantSEQR™ primer sets are available for a wide range of genes involved with genetic disease. Clinical laboratories are interested in the performance of the VariantSEQR™ system in a diagnostic setting, particularly since it can be coupled with SeqScape® v2.1 automated

mutation detection. We performed a blind study on a panel of 76 patients to test the performance of the complete neurofibromatosis type 2 (NF2) gene VariantSEQR™ primer set (34 fragments) and analysed the data using the SeqScape® v2.1 automated sequence analysis/mutation detection software.

The NF2 VariantSEQR™ kit PCR gave robust amplification for most of the fragments (28/34). Sequence data was produced for nearly all the fragments for each patient DNA. After only a single round of repeat amplifications and sequencing only 6 fragments from the whole data set of 76 patients (6/4560) failed to produce any sequence data. However a very large amount of sequence data is generated (18.3kb in one direction) to cover the regions of interest (complete coding and non-coding sequences of the NF2 gene = 7.6kb).

The sensitivity of mutation detection of the VariantSEQR™/SeqScape® protocol observed with our panel (containing 20 mutation positive controls) was seen to be 17/20 mutations (85%) under default analysis conditions. Two of the mutations missed were heterozygous frameshift deletions, and the third a mutation lying within a portion of an exon that repeatedly failed to sequence. Although the NF2 VariantSEQR™/SeqScape® system was able to detect the majority of mutations the inability to automatically detect frameshift mutations is a concern that needs addressing.

P0784. Evaluation of a service for Sorsby Fundus Dystrophy

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Sorsby Fundus Dystrophy (SFD) is an autosomal dominant macular dystrophy. Loss of vision in SFD results from the growth of new choroidal vessels or atrophy of the outer retina typically in the fourth or fifth decades in those affected.

SFD results from gain-of-function mutations in the tissue inhibitor of metalloproteinase-3 (TIMP-3) protein which is encoded by five exons. All six pathogenic mutations reported are located in exon 5 and the intron 4 boundary. There is a very strong founder effect in the UK with the p.S204C missense mutation accounting for the overwhelming majority of positive cases.

We report the results of 18 months of a diagnostic evaluation using bi-directional sequencing of exon 5 of TIMP-3. During this period we have performed diagnostic testing on 24 individuals and predictive testing on 3 individuals with a confirmed family history. Of the 24 diagnostic tests carried out 11 cases carried p.S204C* and in 12/23 no mutation was found.

Additionally we have identified the unclassified variant p.E162K, previously unreported. It segregated with disease and was not present in 600 ethnically matched control chromosomes.

We have demonstrated a demand for diagnostic and predictive testing for SFD from both ophthalmic and genetic specialist centres across the UK. Due to the potential outcomes for the patient, we request that samples are appropriately referred, with informed consent and have a requirement for duplicate samples where predictive testing is requested. All results are delivered within a genetic counselling environment.

*p.S204C (previously Ser181Cys) has been reclassified according to HGVS guidelines.

P0785. Mitochondrial variants in Non Syndromic Hearing Loss

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Mitochondrial mutations have been previously reported in maternally inherited hearing impairment (HI). The most common mutation is

1555A>G usually associated with history of aminoglycosides exposure. In addition, three other mutations causing non syndromic hearing loss NSHL have been reported: 1494C>T in mtRNA12s, 7510T>C and 7511T>C in tRNA-Ser(UCN). To ascertain the contribution of mitochondrial variations to NSHL, we studied 200 unrelated patients included 50 sporadic cases with aminoglycosides exposure and 150 familial cases with maternally inherited HI.

We analysed mtRNA12s, tRNA-Leu(UUR) and tRNA-Ser(UCN) and the flanking regions by DGGE and sequencing.

The mutation 1555A>G was observed in 4 large families and was always homoplasmic. The phenotype was variable ranging from severe prelingual HI to mild progressive postlingual HI. No aminoglycosides administration has been noted in all the families. The families' origin was varied: France, Arab-Palestinian, Madagascar and Pakistan. In addition, we observed 2 French large families with 7511T>C homoplasmic or heteroplasmic. The age of onset and the severity of the HI were variable even in the same family. In tRNA-Leu(UUR) the mutation 3243A>G was founded in 4 families with HI and other clinical symptoms. No known mutation was found in the sporadic cases. In addition, 25 different variations were founded, 20 have been previously reported as polymorphisms and 5 are new variations.

In conclusion, we have identified a known mitochondrial mutation in 6.6 % of familial NSHL. So, it seems justified to screen only large maternally inherited HI families for mitochondrial mutations.

P0786. Mutation search in the DNA1 and DNAH5 genes in 50 Italian families with Primary Ciliary Dyskinesia

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Primary Ciliary Dyskinesia (PCD) is a rare heterogeneous hereditary disorder characterised by immotility of cilia, leading to recurrent pulmonary and upper respiratory tract infections. The infections often result in bronchiectasis, and they can seriously damage the lungs, which may need to be transplanted. Dextrocardia, with or without heterotaxia (situs inversus totalis), is present in about 50% of the patients. If respiratory infections, ciliary dyskinesia and situs inversus are present, the pathology is named Kartagener Syndrome (KS).

We collected 50 unrelated KS/PCD families from all around Italy. Diagnosis was established on the basis of respiratory tract infections, bronchiectasis, electron microscopy of cilia or flagella. Over half of the patients has a dynein arm deficiency. We searched the patients' DNA for mutations in the genes in which mutations have already been described: DNA1, an intermediate dynein chain and DNAH5 an heavy chain axonemal dynein. The analysis of the entire DNA1 gene did not show pathogenetic alterations. In Italy, PCD/KS is almost never due to mutations in the DNA1. The mutation search on the DNAH5 gene was focused on the exons carrying already published mutations. This strategy was necessary because of the size of this gene. Only one couple of brothers shows mutations in the DNAH5 gene: one is the A insertion in exon 32 at nt 5130, the second one is novel it is in exon 34, and changes a Glu in a stop codon. It is possible that the other Italian patients share the same mutated gene/s which is to be individuate.

P0787. Nance-Horan syndrome: results of mutation screening in 19 independent families

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Nance-Horan syndrome (NHS) is a rare X-linked condition characterized by bilateral congenital cataracts with microcornea, distinctive dental anomalies, typical facial features and mental impairment in 30% of male cases.

The NHS gene was identified in 2003 by an Australian group. NHS is a large gene that encodes a 8.7 kb major isoform and a 7.7 kb minor isoform, resulting in predicted proteins, which differ by their N-terminus. The predicted amino acid sequence shows significant homology between several animal species, but no homology with any known protein family. NHS expression is developmentally regulated in a range of embryonic tissues, including the lens, brain, craniofacial mesenchyme, and dental primordia. The function of NHS is unknown but the expression pattern together with the identification of predicted nuclear localization signals support a key role in the regulation of brain, lens, tooth and craniofacial development. By positional cloning and database searching, we simultaneously identified the NHS gene. We found 2 additional mRNA isoforms which differ by their 5' end, both lacking exon 1, and which display tissue-dependent alternative splicing of an additional exon.

We present the detailed results of mutation screening in a series of 19 independent NHS families. All the mutations identified until now are truncating mutations, mostly nonsense or frameshift. They are spread over the gene and there is no consistent genotype / phenotype correlation. There is no significant difference in the clinical picture between the mutated families and those where no mutation was identified.

P0788. Study of MEFV mutations in the Iranian population by means of reverse-hybridization teststrips

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Familial Mediterranean Fever (FMF) is a hereditary inflammatory disorder caused by mutations in the MEFV gene. Carrier rates are known to be high among Turks, Armenians and Arab populations, whereas no data on the frequency and the spectrum of MEFV mutations were so far available from neighbouring Iran.

We have applied reverse-hybridisation teststrips (FMF StripAssay) to simultaneously analyse twelve common MEFV mutations in 208 asymptomatic Iranians from different regions and ethnic groups. The overall frequency of mutant alleles in our study population (15.6%) was moderate compared to Armenia, but exceeded the values known from Turkey and Iraq. The most common variant E148Q was identified in 9.6% of MEFV genes. Five other mutations (P369S, M694V, V726A, A744S, R761H) were observed with lower prevalence.

In addition, we studied the case of an 8 year old boy with short recurrent fever attacks and abdominal pain from a small village in the northwest of Iran. He turned out to be homozygous for MEFV mutation M694V. His parents were M694V/N and M694V/R761H. Several other members of this large family were found to be affected by typical symptoms of FMF and to carry MEFV mutations. Among 30 asymptomatic inhabitants of this village, who consented to participate in our study, we identified six different variants (E148Q, P369S, M680I(G/C), M694V, V726A, R761H) in a total of 13 mutant MEFV genes. Given the high frequency of MEFV mutations in Iran, the awareness for FMF and the availability of testing needs to increase significantly. (oberkanins@viennalab.co.at)

P0789. Screening of GATA4 gene in patients with isolated atrioventricular canal defect

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Atrioventricular canal defect (AVCD) is frequently associated with extracardiac anomalies, and peculiar anatomic patterns are found with distinct genetic syndromes. In particular, the complete form of AVCD, which accounts for 70% of the cases, is associated with trisomy 21 (20%), and deletion 8p23 (40%), while the partial form occurs is some

mendelian disorders. Mutations in *CRELD1* gene has been detected in 6% of isolated AVCD patients, while a missense mutation in *GATA4* gene, mapped to 8p23, was found in a family segregating either atrial or ventricular or AV septal defects. We screened *GATA4* gene in a cohort of sporadic and familiar cases of non-syndromic AVCD. Twenty-two sporadic individuals and nine families, with at least one individual affected by AVCD have been enrolled in this study. Six sporadic and one familial patients had additional heart defects, in particular 5 patients were affected by AVCD-Tetralogy of Fallot. Recurrence of CHDs was concordant in 3 families and discordant in 5. Chromosome and *CRELD1* gene analyses were negative in all individuals. *GATA4* gene analysis was performed by PCR, SSCP and direct sequencing. No mutation was identified in the *GATA4* gene coding region. Our study does not support a pathogenetic role of *GATA4* mutations in AVCD. Although *GATA4* gene haploinsufficiency is considered responsible for cardiac septal defects, either in del8p syndrome and in some non-syndromic patients, present results suggest that *GATA4* mutations are not a common cause of isolated AVCD, arguing genes, other than *CRELD1* and *GATA4*, should be causally related to this defect.

P0790. Molecular studies of USHER 1 patients in France evidence a high genetic heterogeneity

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Usher syndrome is a recessive disorder associating hearing loss (HL) and retinitis pigmentosa (RP). Current classification divides Usher syndrome in three distinct clinical types. Usher syndrome type 1 (USH1) is the most severe form characterized by profound congenital hearing loss, vestibular dysfunction and prepubertal onset of retinitis pigmentosa. To date 5 genes causing USH1 have been cloned: MYO7A, CDH23, PCDH15, USH1C and SANS.

Because no specific phenotype can yet be related to either gene, it is still necessary to plan the screening of several genes before identifying the deleterious one(s). We therefore developed a two-step strategy to perform an extensive study of USH1 genes. In first intention and when possible, we perform linkage studies with microsatellite markers neighboring the different USH1 genes to point the gene(s) to be screened. We then perform mutation analysis by using the Single Condition Amplification/Internal primer method (SCAIP). The latter approach allows entire sequencing of a single gene with a single PCR condition in a reduced time. We screened several dozens of patients for MYO7A and CDH23. The study reveals known mutations for both genes as well as 17 new pathogenic mutations. Since no mutation could be identified in a number of patients, the analysis of the other USH1 genes is underway. Finally, the SCAIP method also identifies a high number of SNPs distributed throughout the genes, thus permitting the building of complex haplotypes for several USH1 genes for each patient. These data will be integrated for further phenotype/genotype correlations.

P0791. Functional characterization of a novel sporadic mutation in Von Hippel Lindau (VHL) gene.

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von Hippel Lindau disease (OMIM # 193300) is a heritable autosomal dominant cancer multisystem cancer syndrome that results from a germline mutation in the VHL gene. Germline mutations in the VHL gene lead to the development of several benign or malignant tumours and cysts in many organ systems. Principal lesions are retinal and CNS haemangioblastomas.

In a screening for germline mutations of VHL patients we have identified a case of germline mosaicism in an affected individual by VHL syndrome without a family history of the disease. The nucleotide alteration consists of an 11bp tandem repeat, located in the VHL promoter region. Analysis of this altered region of the VHL promoter using a computer algorithm revealed the presence of an additional Sp1 and AP2-alfa binding sites.

In order to characterize the potential Sp1 and AP2 binding sites and to investigate the functional consequences of the repeat we performed EMSA and transactivation assay. Moreover the levels of VHL mRNA have been assessed. The result of this analysis will be presented.

P0792. Study of genetic risk factors for cardiovascular disease in the Iranian population by means of reverse-hybridization teststrips

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A number of genetic and environmental risk factors have been found or suspected to predispose to cardiovascular disease (CVD), the term collectively used for disorders of the heart and blood vessels. We have developed a reverse-hybridization assay (CVD StripAssay) for the rapid and simultaneous detection of twelve candidate CVD risk factors (Factor V Leiden, Factor V R2, Prothrombin G20210A, Factor XIII V34L, beta-Fibrinogen -455 G-A, PAI-1 4G/5G, GPIIa L33P, MTHFR C677T, MTHFR A1298C, ACE Ins/Del, Apo B R3500Q, Apo E2/E3/E4). The test is based on multiplex PCR and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. We have applied these teststrips to investigate the prevalence of CVD risk mutations among 208 asymptomatic Iranians from different regions and ethnic groups.

The allele frequencies of mutant Factor V Leiden (1.2%) and Prothrombin G20210A (0.5%) in our cohort were below previously published figures on the population of Tehran (2.7% and 1.5%, respectively; Zeinali et al. 2000). Mutant MTHFR C677T (24.8%) and Factor XIII V34L (14.2%) occurred less frequently than among Europeans, but exceeded the much lower frequencies known from India and most of Asia. The prevalence of mutant MTHFR A1298C in our study population (41.8%), however, was remarkably high. Apo E2 (4.6%) and E4 (5.8%) alleles were observed in relatively low frequencies compared to population studies in Europe and the USA.

Our comprehensive population data should represent a valuable basis for further investigation on the contributions of genetic CVD risk factors in Iran. (oberkanins@viennalab.co.at)

P0793. Prototype Genomic DNA-based Reference Materials for Fragile X and Hereditary Non-Polyposis Colorectal Cancer Testing. On behalf of the Certified Reference Materials for Genetics (CRMGEN) project

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The use of appropriate Reference Materials (RMs) to validate test equipment or methods is an important part of diagnostic systems however, currently no Certified Reference Materials (CRMs) are available for molecular genetic testing. The CRMGEN project (EC Contract: G6RD-CT-2001-00581) aims to develop CRMs for a range of diseases and by doing so, develop the methodology to produce CRMs for any molecular genetic test. Four formats of RMs are being investigated by the project's producing partners: Cultured cell lines (Leuven), Recombinant DNA fragments (Leiden), PCR products (Dublin) and Genomic DNA (Manchester). Initially, Manchester has concentrated on developing RMs relevant to testing for Fragile X syndrome and Hereditary Non-Polyposis Colorectal Cancer (HNPCC). Samples have been collected from 13 Fragile X and 6 HNPCC patients under informed consent. Stable cell lines have been established from these samples and stored at the National Institute for Biological Standards and Control (NIBSC). Six HNPCC and 5 of the Fragile X cell lines were selected for the development of prototype RMs. Four methods for large scale DNA extraction were developed and tested in field trials. From the results, it was decided that a batched phenol-chloroform methodology would be used in future genomic DNA-based RM production. The 11 prototype RMs were fully characterised and distributed for field trialling, in conjunction with the European Molecular Genetics Quality Network, to 54 Fragile X and 43 HNPCC testing laboratories. The results indicate that genomic DNA-based RMs perform well in a diagnostic laboratory setting.

P0794. Clinical and biochemical characteristics in patients with mitochondrial T8993C/G mutations

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Deficient complex V activity has been reported in patients carrying the T8993G point mutation in the mitochondrial ATPase 6 gene. However, the effect of the T8993C mutation on complex V function in muscle has not yet been described. We retrospectively analyzed the biochemical, clinical and histological data of eleven patients, five of which carried the maternally inherited T8993C and six carrying the T8993G mutations. The percentage of heteroplasmy was higher than 95% in muscle and in blood. We compared the ATP production from pyruvate oxidation and the activity of complexes I-V measured in fresh muscle biopsy samples in the two patient groups. We also evaluated the assembly-process of complex V in muscle tissue. In nine patients a decreased ATP production was detected, and complex V was deficient in all children. The activity of the respiratory enzyme complexes I-IV was normal in nine patients, whereas complex I and III were deficient in two. All patients had an early clinical presentation with muscle hypotonia, severe extrapyramidal dysfunction and Leigh disease demonstrated by the cranial MRI. A slower clinical progression and more frequent senso-neural involvement were noted in the patient group carrying the T8993C mutation. No obvious difference was found in the biochemical parameters. We couldn't find any correlation between the degree of complex V deficiency and the severity of the phenotype. We confirmed an assembly defect in our patients. This is the first report of decreased activity and delayed assembly of complex V in patients with T8993C mutations measured in muscle tissue.

P0795. PABPN1 gene point mutations associated with oculopharyngeal muscular dystrophy (OPMD) and a novel mechanism for triplet repeat expansion.

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant late onset neuromuscular degenerative disease characterised by proximal muscle weakness, ptosis and swallowing difficulty. The underlying cause is a triplet repeat expansion of 2 - 7 additional base triplets in a repeat sequence in exon 1 of the polyadenine binding protein nuclear 1 gene (*PABPN1*). This results in an increase in length of from 10 to 12 - 17 residues of a polyalanine tract in the *PABPN1* protein and is associated with deposition of characteristic nuclear filament inclusions in skeletal muscle fibres. *PABPN1* is ubiquitously expressed and binds to the polyadenine tail of pre-mRNAs, being involved in regulation of their formation and length.

The triplet repeat expansion mutation has been the only OPMD mutation documented to date, however we have identified two point mutations in *PABPN1* associated with OPMD and an OPMD like phenotype. Both are miss-sense mutations close to the 3' end of the *PABPN1* polyalanine repeat sequence. In case A, who has the typical OPMD phenotype, a c.35G>C/ p.Gly12Ala mutation generates a polyalanine tract of 13 contiguous residues by the change of only a single base. This represents a previously undescribed mechanism of triplet repeat expansion. Cases B1 and B2 have the mutation c.50G>C/ p.Arg17Pro mutation and an early onset OPMD like phenotype but with no evidence of intranuclear inclusion bodies.

P0796. In frame 21 bp exonic deletions in the PQBP1 X-linked mental retardation (XLMR) gene : pathogenic mutations or rare variants of uncertain effect ?

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Mutations in the PQBP1 gene, coding for the polyglutamine-binding protein 1, a putative transcription factor, have recently been identified in 8 families with syndromic or non syndromic X-linked mental retardation (XLMR). Clinical features frequently associated with the mental retardation are microcephaly and/or short stature and in a smaller proportion spasticity, small testes, anal atresia or stenosis, cardiac defects, ocular coloboma, cleft palate and other craniofacial abnormalities. The predominant mutations detected (5/8) affect a stretch of six AG dinucleotides in the polar-amino-acid-rich domain (PRD), causing frameshifts in the fourth coding exon. Further studies identified another deletion in exon 4, an insertion in exon 5, and a missense mutation in exon 3.

We searched for PQBP1 exon 4 frameshifts in a cohort of mentally retarded males initially referred with a clinical description of at least one of the following criteria : microcephaly, short stature, spastic paraparesis or a family history compatible with XLMR; and also in a large cohort of mentally retarded males not selected for specific clinical features or familial history.

We identified a novel frameshift mutation (a 23bp deletion) in two half-brothers presenting specific clinical features. We also performed a molecular prenatal diagnosis in this family.

We additionally detected in three independent probands two different in frame deletions of 21bp, deleting one of 5 copies of an imperfect 7 aminoacid repeat. The nature of the mutations, the lack of consistent associated clinical features, and the borderline cognitive impairment phenotype showed by some patients, lead us to discuss their pathogenic significance.

P0797. Complex I deficiency in Iranian multiple Sclerosis patients

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Multiple sclerosis (MS) is an 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) on intact mitochondria isolated from fresh skeletal muscle in MS patients(n=10)and control subjects(n=11) was performed. Common deletion and deletion also was tested in mtDNA of MS patients. Our findings showed that complex I activity 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 disorder is due to predominant maternal transmission of MS in affected parent-child pairs, pathoetiological role of respiratory chain dysfunction in multisystem disorder 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 patogenesis of MS.

P0798. Molecular Diagnosis of the Classical Type of Ehlers-Danlos Syndrome (EDS Type I/II)

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Major diagnostic features of the classical EDS type are hyperextensible skin, tissue fragility with widened atrophic scars, and joint hypermobility. Approximately 50% of classic EDS cases result from abnormalities in type V collagen, a molecule that predominantly occurs as a heterotrimer of two a1 (V) and one a2 (V) chains encoded by the COL5A1 and COL5A2 genes. The clinical diagnosis of classic EDS can be strengthened by abnormal ultrastructural dermal architecture recognized by characteristic findings in electron microscopy.

We report on the molecular analysis of 35 patients with the clinical diagnosis of classic EDS. Electron microscopy of a skin biopsy

preceded molecular genetic analysis in 17 cases. Mutation screening of all coding exons of the COL5A1 and the COL5A2 gene by direct sequencing of leukocyte DNA has been finished in 22 patients, 11 of whom had additional ultrastructural findings. Most likely pathogenic mutations were identified in 10/22 patients (45%); in five patients transmission electron microscopy revealed typical composite collagen fibrils with enlarged "flower-like" cross sections and rope-like longitudinal sections. All mutations affect the COL5A1 gene and none has been previously reported. Seven are translation terminating composed of four frameshift, two splice site and one nonsense mutation. Two missense changes affect a glycine residue and a conserved arginine residue in the triple-helical domain of the proa1 (V) chain, respectively. One patient carries a one amino acid in-frame deletion. Our detection rate of 45% is in accordance with previously reported molecular genetic studies and supports further genetic heterogeneity in classic EDS.

P0799. Novel and recurrent mutations in the ATM gene in patients with classical ataxia-telangiectasia (AT)

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The gene for ataxia-telangiectasia (MIM 208900), ATM, spans about 150 kb of genomic DNA. ATM mutations are found along the entire gene, with no evidence of a mutational hot spots. We screened the ATM gene using an optimized denaturing high performance liquid chromatography (DHPLC) technique that detected all previously known mutations in PCR segments being analyzed. The screening was performed in 22 unrelated AT patients, and allowed us to identify 38 of the 44 expected mutations. Therefore, the efficiency of mutation detection by optimized DHPLC was approximately 86%. Sixteen (73%) of the investigated patients were compound heterozygous, and six (27%) were homozygous for an ATM mutation. Among the 38 mutations identified, we observed 6 nonsense alleles (16%) and 26 (68%) frameshift alleles, including 16 deletions, one insertion, 7 out-of-frame exon skipping events and one homozygous duplication. Thus, as many as 32 (84%) alleles caused, directly or indirectly, a premature termination codon (PTC). Two alleles (5%) caused in-frame exon skipping. In addition, 4 (11%) missense mutations were also observed. Mutations were scattered across the whole coding sequence of the ATM gene, and 13 of the mutations were found to be novel according to the ATM Mutation Database. For the three mutations [c.3576G>A (K1192K), c.3802delG and c.7517_7520delGAGA] recurrently detected we defined the associated haplotypes in order to investigate whether they are ancestrally related or hot spots. The identification of ATM gene mutations appears important for understanding the molecular basis of the disease, and is essential for diagnosis and genetic counseling.

P0800. Characterization of a fourth form of Autosomal Dominant Hypercholesterolemia in a French family

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Autosomal Dominant Hypercholesterolemia (ADH) is characterized by isolated elevation of LDL cholesterol and associated with high risk of premature cardiovascular complications. Over 1000 mutations in the *LDLR* gene (Familial Hypercholesterolemia, FH) and 5 mutations in the *APOB* gene (Familial Defective Apolipoprotein B100) have been implicated. We recently reported further genetic heterogeneity related to missense mutations in the *PCSK9* gene. We now report a large French ADH family in which involvement of the *LDLR*, *APOB* and *PCSK9* genes was excluded. We named the pathology "HCHOLA4". Our aim was to identify the disease-causing gene and to define the associated pathophysiology.

A whole-genome scan, using 232 polymorphic microsatellite markers, located the *HCHOLA4* gene at 16q22.1. Regional haplotype construction allowed identification of a 5.9 cM critical interval between

markers D16S3043 and D16S3018. Regional and functional candidate genes were tested by sequencing but no causal mutation was detected.

In vivo kinetics of apolipoprotein B100-containing lipoproteins, conducted in 2 affected members, mainly showed substantial decrease in LDL catabolism compared to normocholesterolemic individuals. A Q-PCR analysis of SREBP2 (Sterol Responsive Element Binding Protein) and 2 of its target genes (*HMGCR*, *LDLR*) in EBV-transformed lymphoblasts of one affected member showed a huge decrease of expression versus normocholesterolemic an FH patients in standard culture conditions.

In conclusion, we identified a French family with a fourth form of ADH linked to 16q22.1 whose LDL elevation is due to a substantial default in LDL catabolism and characterized by extremely low basal expression of *SREBP2*, *HMGCR* and *LDLR* genes in lymphoblasts.

P0801. Rapid heteroduplex scanning of the GALT gene using dried blood spot DNA.

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Classical galactosemia results from defects in galactose-1-phosphate uridyl transferase gene (GALT) and is identified by newborn screening. Screening utilizes the dried blood spot (DBS) to assay total galactose and/or GALT enzyme activity. Screening identifies some carriers and some specimens with partial deficiencies (so-called D/G compound heterozygotes). Screening labs use DNA from DBS to assay common mutations but DBS is considered an inadequate source of DNA for comprehensive gene analysis. Two normal specimens and thirty-eight specimens testing beyond cut-offs in newborn screening were assayed over the GALT gene using dye-binding/high-resolution thermal denaturation with DNA from the newborn DBS. Exons were assayed as single amplification products included coding and intron sequence critical to splicing. PCR included the dye LCGreen™. Post-PCR dye-saturated product was melted in the HR-1™ instrument. Samples with aberrant melt-profiles were recovered for DNA sequencing. Nine classical galactosemia, 16 carriers, and 11 D/G compound heterozygotes were identified. No mutations were found in 2 specimens with elevated galactose and normal enzyme activity suggesting galactose epimerase deficiency. In one likely carrier no mutation was observed. The following mutations were observed: fs D39X (c17-18 del CC), ΔD97 (c289-291 del AAC), R67H, S135L, T138M, M142K, F171S, Q188R, S192N, L195P, R231C, Y251S, R263G, T284N, M298V, Y323C, R333W, Q344K. Comprehensive analysis of GALT used 11ul of the 60ul of DNA obtained from a 3.2mm punch showing the DBS is useful for comprehensive gene analysis. The protocol including preparing DNA, PCR, melting, and preparing specimens with aberrant melting profiles for sequencing is completed in 4 hours.

P0802. Screening for Wilson Disease: 2nd tier molecular analysis of ATP7B

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Wilson Disease (WD, OMIM#277900) is an autosomal recessive disorder of copper metabolism secondary to mutations in the ATP7B gene. Presentation is characterized by hepatitis, cirrhosis, or neuropsychiatric symptoms. Early detection of WD averts irreversible tissue damage thus prospective screening is proposed using reduced ceruloplasmin as the biochemical genetic marker. Among candidate patients with low CP, there are no secondary biochemical or clinical markers, thus 2nd tier evaluation of ATP7B is being investigated. Analysis of ATP7B applies dye-binding/high-resolution thermal denaturation to identify regions of sequence aberration to target DNA sequencing. Coding and critical splice site regions are amplified in the presence of the saturating dye LCGreen™ Plus. High-resolution thermal denaturation of dye saturated amplification product used the LightScanner™ instrument. Normal profiles were established for

each fragment and those deviating from normal were recovered for sequence analysis. Among 16 patients, mutations were observed in exons 8-20, the most frequently observed were R778L and H1069Q. Other mutations identified include: M769I; A874V, c2697-2723 del IVKLVEEAQ; G988R, G1035V; L1083F, 3400 delC, I1148T, N1270S, Q1351X. Polymorphisms were observed in 11/16 specimens and include, R725R, L770L, K832R, R952K, T991T, V1140A, IVS12 -13 G>T, IVS18 +6 C>T. Amplification of all regions uses a common PCR protocol and melting is performed in the PCR plate. Melting is not destructive and LCGreen Plus does not interfere with subsequent sequencing thus samples are recovered from the reaction plate for sequencing. Dye-binding/high-resolution thermal denaturation is a cost effective means to assay ATP7B in candidate patients identified by prospective screening using reduced ceruloplasmin.

P0803. Origin of *de novo* KCNJ11 gene mutations causing neonatal diabetes

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The KCNJ11 gene encodes the Kir6.2 subunit of the beta-cell ATP-sensitive potassium channel. We have recently identified KCNJ11 mutations in 23 families with neonatal diabetes. Nineteen (83%) of the probands are apparently sporadic cases. In two families a second generation was affected and microsatellite markers were used to determine the parental origin of the mutation. A third family includes two affected half-brothers whose unaffected father is presumed to be a germline mosaic. For 13 families DNA was available from parents and informative polymorphisms were present in 8. Allele specific PCR for a heterozygous polymorphism (E23K, A190A, I337V) or the mutation was used to amplify across the mutation or polymorphism and phase determined by sequencing.

Seven mutations had arisen on the paternal allele and one was maternal in origin. There was no evidence of an association with advanced paternal age. Real-time PCR analysis of lymphocyte DNA found no evidence of somatic mosaicism in the father presumed to be a germline mosaic. The maternally derived KCNJ11 mutation showed a low level (2.8%) of somatic mosaicism, suggesting the likelihood of germline mosaicism. It is important to consider the possibility of germline mosaicism when counselling parents of an apparently sporadic case for recurrence risks.

P0804. Cyp21 gene analysis in 50 patients with 21-hydroxylase deficiency in Iran

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency is one of the most common (1 in 10,000 to 1 in 15,000) autosomal recessive disorders. The aim of this study was to assess the frequencies of the eight most common mutations in the CYP21 gene in individuals with 21-hydroxylase deficiency. We applied allele specific polymerase chain reaction previously, as described by RC Wilson et al., to detect the eight most common mutations in the CYP21 gene. Fifty unrelated patients with symptoms of classical CAH (salt wasting or simple virilization) were studied. We could detect overall 66% of the mutations. The most frequent mutations were found to be I2G (29%) and an 8 bp deletion in exon 3 (10%). The frequency of other alleles was: I172N (8%), V281L (3%), exon 6 cluster (I236N, V237E, and M239K) (4%), Q318X (7%), R356W (5%), and we did not detect the P30L mutation in any of our patients. The frequency of mutations did not differ substantially from frequencies of other countries.

P0805. Genotype-Phenotype correlations of GJB2 mutations

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In this study, we analyzed the genotype-phenotype correlations in the Iranian GJB2-related deaf population. Included in this study were 147 patients who met the defining criteria for autosomal recessive non-syndromic hearing impairment. Of these, 95 patients (65%) were homozygous for the 35delG mutation and 25 patients (17%) carried the 35delG mutation *in trans* with another GJB2 mutation. The next two most prevalent mutations were W24X, present on 17 chromosomes (5.8%) and -3170G>A, present on 14 chromosomes (4.8%). In the entire group, severe-to-profound deafness was seen in 83.8% of the cases, including 92% of those homozygous for the 35delG mutation. Interestingly, mild hearing loss was found in a subject homozygous for 35delG, which is unusual. All persons segregating missense mutations, whether homo- or heterozygous, had severe-to-profound hearing loss.

P0806. Screening for CFTR mutations in Southern European Countries

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The gene for cystic fibrosis was identified in 1989 and this together with the emerging technology for mutation detection heralded a new dawn for the diagnosis of the disease. Genotypically, the frequencies and types of mutations vary according to the geographic and ethnic origin of the population studied. A total of more than 1200 mutations have been identified worldwide with F508del, showing a North-to-South Europe decreasing frequency gradient (90-30%) while the percentage of non-F508del chromosomes associated with CF increases. Mutation screening is especially difficult with commercially available kits in the Mediterranean region ($\leq 75\%$ of CF allele detection). For the Greek population 80 different mutations (20 specific to our population) account for 91% of CF genes generating 103 different genotypes. For the Spanish population more than 75 different mutations represent 90.2% of the CF alleles. The south of France shows allelic heterogeneity ranging from 75 mutations to about 30 accounting for 97.6% of CF alleles. In southern Italy 43 mutations detect 91.5% of CF mutated chromosomes. Identification of the alleles that are common in the Mediterranean region will help in the design of specific assays. Whole gene scanning methodologies such as DGGE or DHPLC combined with sequencing are currently the methods of choice, but moving towards a more high-throughput array technology is desirable. The advantage of the micro-arrays is flexibility in adding additional ethnic-specific mutations and the Nanogen NanoChip™ platform for which we have developed assays to cover $>85\%$ of CF alleles in the Greek population, offers such a possibility.

P0807. Contribution of GJB6 large deletion to the hereditary deafness genetic load in the Iranian population

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Mutations in the gene that encodes the gap-junction protein connexin 26 (GJB2) at the DFNB1 locus on chromosome 13q12 are the major cause of autosomal recessive non-syndromic sensorineural deafness (ARNSD) in many different populations. A second gap-junction gene, GJB6, also localizes to the DFNB1 interval. Interestingly, the encoded protein connexin 30 is expressed in the same inner-ear structures as connexin 26 and both connexins are functionally related. The importance of GJB6 to normal hearing has been confirmed by the identification of a large deletion (Δ (GJB6-D13S1830)) involving the first two exons and a part of third exon of GJB6 and a large region of the upstream sequence in persons with ARNSD. Homozygotes for this deletion and compound heterozygotes carrying Δ (GJB6-D13S1830) and a deafness-causing allele variant of GJB2 have severe-to-profound congenital deafness. The aim of this study was to evaluate the contribution made by this deletion to the ARNSD genetic load in the

Iranian population. One hundred and fifty four probands with ARNSD from various regions of the country were screened for this mutation using polymerase chain reaction (PCR) primers that amplified the breakpoint junctions of the deletion. Among them, one hundred and sixteen patients were deaf probands with normal *GJB2* alleles and the remaining 38 were heterozygote for only one *GJB2* mutation. None of patients screened for Δ (GJB6-D13S1830) was shown to carry this deletion, suggesting that this mutation is not a common cause of deafness in Iran.

P0808. Analysis of association of candidate gene polymorphisms with decreased bone mineral density

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Osteoporosis is a complex disease characterized by decrease of bone mineral density (BMD) and a microarchitectural deterioration of bone structure, leading to a higher susceptibility to fractures. In recent years different studies have suggested a major genetic contribution to bone mass determination and to the development of osteoporosis. Several candidate genes were identified but the role of each in the development of osteoporosis is not clearly determined. We started to examine the association of candidate gene polymorphisms with decreased bone mineral density and osteoporotic fractures in patients from different ethnic groups from Bashkortostan. We examined the *Spl* polymorphism in the collagen type 1A1 (*COL1A1*) gene, the *FokI* polymorphism in the vitamin D receptor (*VDR*) gene, and the *XbaI* and *PvuI* polymorphisms in the estrogen receptor (*ER*) gene in patients with osteoporosis and in a control group. Analysis of the *Spl* polymorphism in *COL1A1* revealed an increased frequency of the ss genotype (0.033) in patients with osteoporosis compared with controls (0.066); this difference was not statistically significant ($p>0.05$). We have found association of ss genotype with osteoporosis (OR=1.2). We also have revealed significant differences between Bashkirs and Russians ($p=0.02714$), and between Tatars and Bashkirs (0.00268). For the *XbaI* and *PvuI* polymorphisms in the *ER* gene we saw no significant differences in the allele and genotypes frequencies in patients with osteoporosis in compared with healthy individuals ($p>0.05$). Thus, we can suggest that it is necessary to take into account the ethnicity of individuals in association studies.

P0809. An international network for genetic diagnostics GENDIA

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Molecular diagnostics for genetic diseases remains a challenging problem. For many diseases local tests are not available, or incomplete, expensive and slow. The most important bottlenecks for cost-effective molecular diagnostics are the rareness of genetic diseases in general and the private nature of most mutations. This precludes cost-effective set up of molecular diagnostics on a regional or even national level. To facilitate genetic diagnostics a worldwide network of diagnostic labs was organised, existing of many referral labs, 40 expert test labs, and 1 central lab that accepts all samples and issues all results. This network is called GENDIA (for GENetic DIagnostics). GENDIA now offers more than 600 genetic tests (www.GENDIA.net), including molecular analysis of more than 400 genes.

P0810. Determination of Alpha Thalassemia mutations spectrum in Iranian population

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Alpha-thalassemia is the most common inherited hemoglobin disorder worldwide, it is caused by a variety of deletional and non-deletional α -globin mutations, leading to a reduction or complete absence of gene expression.

It is observed in high frequencies throughout southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area.

In this study we have tested 421 Iranian individuals, randomly chosen from a pool of patients with low MCV, low MCH, normal or slightly

reduced Hb levels, normal HbA₂.

Mutations were identified in 284 of cases; 166 were α 3.7/aa (39.43%), 33 were α 3.7/ α 3.7 (7.83%), 14 were Med/aa(3.32%), 4 were α 3.7/ α 4.2(0.95%), one was α 3.7/Med(0.23%), 22 were α 4.2/aa (5.22%), one was α 4.2/ α 4.2(0.23%), one was α 4.2/Med(0.23%), 4 were α 20.5/aa(0.95%), 13 with point mutation acsa/aa (3.09%), and also 14 cases had polyA mutations α PAa/aa (3.32%).

We also found other mutations α (-5nt) α /aa, α -22 α /aa(0.23%), α (+14) α /aPA and α (+14) α /aa in 7 cases.

Two other cases had α 14 α /aa polymorphism that is the most common polymorphism and we also detected a novel mutation α (+14;Cd20)/aa in 2 cases(0.47%). In 137 individuals none of these mutations were found. Our study shows that α globin mutations were found in 66.75% of individuals that we have tested and the most common mutations of α -thalassemia are single or double α -globin gene deletions α 3.7.

P0811. FLJ23451, A novel gene note expressed in the 2P16 deletion syndrome

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We identified Bedouin patients presenting a unique recessively inherited syndrome, with broad clinical manifestations: cystinuria, mental & growth retardation, hypotonia, facial dysmorphism and mitochondrial respiratory chain dysfunction presenting as reduced activity of all mitochondrial encoded respiratory chain enzymatic complexes. Our previous study identified the molecular basis of this syndrome as a homozygous deletion of 179,311 bp on chromosome 2p16, and thus was subsequently named the 2p16 deletion syndrome. Further studies to define the transcription content of this interval showed that it includes 4 protein coding genes: type I cystinuria *SLC3A1*, protein phosphatase 2C β , two unidentified genes *KIAA0436* and *FLJ23451* and three transcripts without, or with very short, open reading frames. The *FLJ23451* gene has its first exon in the deletion and thus is not expressed in patient's cells. Bioinformatics investigation suggests that *FLJ23451* belongs to a family of methyltransferases. It is highly conserved during evolution, ubiquitously expressed- including the tissues affected by the syndrome and our preliminary results suggest nuclear localization. We hypothesize that the absence of this gene in the patients may have a major contribution to the clinical presentation of the patients.

P0812. Growth retardation associated with iron-sulfur enzymes deficiency in frataxin deficient stable murine fibroblasts cell lines

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Friedreich ataxia (FRDA), the most common recessive ataxia, results from a generalized deficiency of mitochondrial and cytosolic iron-sulfur (Fe-S) protein activity due to a partial loss of function of frataxin, a mitochondrial protein involved in Fe-S cluster (ISC) biosynthesis. Oxidative stress has been proposed to be involved in the pathogenesis of FRDA, leading to the use of antioxidants for therapy. However, to this date, FRDA remains a devastating disease for which there is no cure.

Our key objective is to find an effective treatment for FRDA using cellular and mouse models that will then be transferred to the clinical research field. Conditional knockout mice already exist but may be very expensive models for drug assay. Therefore, an initial screening should be undertaken first in cellular models.

The absence of spontaneous phenotype and the genetic heterogeneity in FRDA patient cell lines make them unsuitable for drug screening. We have recently developed a murine fibroblast cell model based on antisense strategy using a ribozyme which shows highly reduced levels of frataxin reproducing the quantitative defect found in patients. These cell lines exhibit a proliferation defect associated with an ISC enzyme deficit. This model is the first stable cellular model for FRDA that shows spontaneous phenotype without exogenous oxidative insult, and is therefore a key model for drug screening.

We therefore propose to screen a library of chemical compounds on this novel cellular model (using high-throughput screening technology)

in order to identify novel pharmacological compounds that may potentially work in combating the disease.

P0813. Hereditary ataxias - differential diagnosis and comorbidity

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The hereditary ataxias are a group of genetic disorders characterized by slowly progressive incoordination of gait and often associated with poor coordination of hands, speech, and eye movements. The differential diagnosis of disorders that include ataxia now includes a new neurodegenerative disorder - the fragile X tremor/ataxia syndrome (FXTAS). The symptoms are very similar: intention tremor and gait ataxia and/or mild parkinsonism, peripheral neuropathy, lower limb proximal muscle weakness, short-term memory loss, executive function deficits, cognitive decline, and autonomic dysfunction.

Potentially FXTAS could be a relatively frequent cause of adult onset progressive ataxia or tremor. According to the literature, the premutation, an expansion of 50 to 200 CGG repeats in the *FMR1* gene, has a prevalence in the general population of approximately 1 per 700 men and 1 per 250 women.

In the last five years we have created a patient register and DNA bank of more than 400 samples from probands with spinocerebellar symptomatology. We demonstrate our experience with diagnosis of spinocerebellar ataxias at the DNA level and note a possibility of comorbidity in a case of a patient with DNA-verified diagnosis of late-onset Friedreich's ataxia plus a premutation in the *FMR1* gene.

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P0814. Study of genetic, epigenetic and expression events associated with MECP2 in patients with Rett syndrome: an attempt to validate MECP2 as a single underlying cause of Rett

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Rett syndrome is a progressive neurodevelopmental X-linked disorder associated with mutations in MECP2 gene. In the course of our experience at the Central Rett Clinic at the Sheba Medical Center, offering clinical and molecular diagnoses of Rett and clinical follow-up, we have identified 70 classical and atypical patients. This entire cohort has been diagnosed under standardized phenotypic criteria and using molecular indicators of MECP2 deficiency including sequence analysis of MECP2 coding region and intron-exon junctions, MLPA deletion detection assay and evaluation of X chromosome inactivation. Following, we identified 62 patients with known and novel mutations involving MECP2 coding region. In attempt to address the need of diagnosis of patients with clinical yet not molecular indication of Rett, we developed an alternative approach based on direct estimates of expression levels of the two MECP2 isoforms in peripheral blood. Thereby, we identified additional 5 patients with lower blood MECP2 expression that may indicate presence of mutations in the regulatory elements of MECP2 gene. Two of these patients were further detected with a novel splice site mutation involving the 2nd alternatively spliced exon of MECP2 that potentially leads to an imbalance between the two MECP2 isoforms. Bioinformatic analysis of the non-coding MECP2 introns, 5'- and 3'-UTRs, revealed several highly conserved elements associated with RNA splicing or transcription mechanisms in other genes. We also detected MECP2 overexpression in several patients with missense mutations, which overlaps with recent findings of a neurodevelopmental delay phenotype on the background of MECP2 overexpression in a mouse animal model.

P0815. Forteen year experience of prenatal diagnosis of Thalassemia in Iran

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For fourteen years Iranian scientists have worked to develop a national thalassemia prevention program. Historically abortion was considered unacceptable in Iran; however, intensive consultations led to the clerical approval of therapeutic abortion of cases with major type of β-thalassemia in 1997, and a nation-wide prevention program with screening, counseling and prenatal diagnosis networks has been developed. This paper reports the experience from one of the two national prenatal diagnosis reference laboratories. From 1990 to 2003 we performed a total of 906 prenatal diagnoses from 718 families at risk for thalassemia. Direct and indirect mutation detection methods were applied for all cases. In total, 22 mutations were tested routinely and an additional 30 rare mutations were identified. 208 fetuses were found to be normal, 215 fetuses were major, and 435 fetuses were trait. In 40 cases we only defined one allele. We were unable to provide 8 cases with any diagnosis, corresponding to 0.9%. Our data supports the functionality of Iranian β-thalassemia prevention program. The success of this system in Iran as a multiethnic and Islamic-based country would mean that it might be applied as an adaptive system for neighboring and other Islamic countries.

P0816. Deletion of DMD exon 16 is not associated with disease.

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Duchenne muscular dystrophy (DMD) is one of the most common inherited neuromuscular diseases, affecting 1 in 3,500 males. It is an X-linked disorder caused by mutations in the *DMD* gene. Covering 2.4 Mb, *DMD* is one of the largest human genes. The gene has 79 exons encoding a 14kb mRNA. Mutations leading to a truncated protein cause the severe phenotype of DMD, whereas mutations retaining the mRNA reading frame cause the more benign phenotype of Becker muscular dystrophy (BMD). Knowing the exact mutation in a patient is therefore prognostic and an important part of the diagnostic tool of these diseases.

By the traditional multiplex PCR one of our healthy male control DNA samples was found to harbour a deletion of exons 16 and 17 predicted to give rise to an out-of-frame mRNA. MLPA analysis indicated, however, only a deletion of exon 16. Closer analysis showed that the deletion covered part of intron 15, exon 16 and almost the entire intron 16. The person is completely healthy, has normal muscle morphology and creatine kinase level. The deletion was also present in DNA from a muscle biopsy, excluding mosaicism as an explanation for the phenotype. We conclude that a deletion of exon 16 of *DMD* does not interfere with the normal function of dystrophin.

P0817. Interleukin-1b gene and receptor antagonist gene polymorphisms in patients with multiple sclerosis

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This study was done to elucidate the effects of interleukin (IL-1) gene polymorphisms on multiple sclerosis (MS).

We evaluated IL-1b (a C/T transition at -511 in promotor) and (+3953 C/T in exon 5) and IL-1 receptor antagonist (IL-1RA) (a variable-number repeat in intron 2) gene polymorphisms in unrelated 114 patients of MS and 166 healthy controls. No significant differences were observed in the allelic frequencies of the IL-1b promoter, IL-1b exon 5 and IL-1RA genes between patients with MS and healthy control subjects. But the frequencies IL-1b (-511) T/T genotype was higher in patient MS with optical breach then controls (38.46% vs. 21.08%; P=0.036; OR=2.33,

CI=1.11-4.92).

Knows, IL-1b (-511 C/T) gene polymorphisms may influence protein production. Were found the IL-1b gene polymorphisms to play a role in the development of clinical particularity MS. Further studies are necessary to determine the biological significance of these findings in relation to susceptibility or severity of the disease.

P0818. Molecular screening of SRD5A2 gene on ambiguous genitalia patients in Taiwan

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Background: Steroid 5 α -reductase(SRD) reduces testosterone to dihydrotestosterone. The genital phenotype of SRD deficiency is heterogenous, ranging from complete female to sexual ambiguity, hypospadias with scrotal bifida, and isolated micropenis. Several different mutations leading to SRD deficiency have been identified in the 5 α -reductase type 2 gene(SRD5A2). In order to understand the extent of SRD5A2 mutation in Taiwan, we analyzed SRD5A2 gene on XY patients with ambiguous genitalia.

Methods: Fifty-five XY patients with either complete female, sexual ambiguity, hypospadias with scrotal bifida, or isolated micropenis were enrolled in this study. The patients distributed from neonate to puberty teenager. Except twelve prepupal patients refusing the HCG test, the others showed normal basal testosterone. Thirty-one had completed the HCG test. The coding region of SRD5A2 gene was amplified by PCR and followed by automatic sequencing. PCR-RFLP was used for mutation confirmation.

Results: The analysis revealed three disease-causing mutations from six unrelated patients, including five homozygous (one G66R, one D95H and three R227E) and one heterozygous (R227E/?). The three cases of homozygous R227E and one homozygous D95H presented as micropenis. The rest two showed hypospadias with scrotal bifida. All parents denied consanguinity. We also noticed that the hot spot R227E accounted for the 7 out of 12 mutation loci.

Conclusion: We found that SRD5A2 mutation might cause micropenis and hypospadias in Taiwan. Most patients showed homozygous mutation though no consanguinity was noted. One hot spot(R227E) was discovered. Further polymorphism study is needed to clarify the founder effect for R227E mutation in Taiwan.

P0819. Phenotype-genotype correlations in 11 Spanish RDH12 mutated families

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INTRODUCTION: Retinitis pigmentosa (RP) is a group of hereditary disorders, which causes retinal dystrophy (RD) and leads to blindness. Leber Congenital Amaurosis (LCA) is a congenital and severe form of RD. The RDH12 gene encodes a retinol-dehydrogenase, a protein of the visual cycle. Mutations in this gene are a cause of severe early-onset RD.

PATIENTS AND METHODS: 22 LCA and 311 unrelated Spanish RP (210 autosomal recessive and 101 sporadic) families were studied. They were ophthalmologically studied and classified into 3 categories: 149 severe, 95 moderate and 66 mild. The RDH12 gene mutation screening was performed by dHPLC followed by sequencing.

RESULTS: Eleven mutated families were found. All of them were severe and early onset RD patients (1 LCA and 10 severe RP). Three mutations were only found among Spanish cases and 2 of them were recurrently seen. L99I (previously reported in a French LCA-family) was present in 5 families (3 homozygotes and 2 double heterozygotes) It was associated to 0-3yr onset RD with nystagmus, nictalopia, visual field impairment and photophobia, followed by early blindness, characteristic fundus (granular black and white pigments

and affected macula) and very early abolished ERG. T155I was found homozygotously in 2 families. Affected patients showed early onset and severe RD with blindness before 40yrs. Four families were heterozygous for 4 mutations.

CONCLUSIONS: Our results suggest that there are 3 founder Spanish mutations (L99I, T155I, c.102ins4) and RDH12 mutations are always associated with a severe RD phenotype, being responsible for 3-4% of early-onset RD in Spain.

P0820. Prevalence of two common mutations and Arylsulfatase-A pseudodeficiency alleles in patients with metachromatic leukodystrophy in Egypt

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Metachromatic Leukodystrophy (MLD) is a neurodegenerative lysosomal storage disease resulting from deficiency of activity of the enzyme arylsulfatase A (ASA). **Objective:** To investigate the efficiency of some biochemical parameters in the diagnosis and to identify the molecular basis of MLD in Egyptian patients. A total of 52 patients presenting with clinical manifestations of MLD were studied using quantitative determination of leucocytic ASA, inorganic sulfates and sialic acid, their ages ranged between 1.5- 4 yrs. Mutations in the ASA gene were identified by PCR amplification and restriction enzyme analysis using BstN1 for detection of splicing mutation at +1 in intron 2 (I) allele, Aci for missence mutation in exon 8, and Bsr1 (glycosylation) site and Rsa1 (poly A allele) for detection of pseudoalleles (P). **Results:** A total of 16/52 (30.7%) were diagnosed as having ASA deficiency. Estimation of sulfates had no significant value while sialic acid was significantly higher in leucocytes of affected cases than controls. It was observed that (7/16) 43.8% of patients were homozygote for the (I) allele, 6/16 (37.5%) were double heterozygote for (I/P) alleles, none of the patients had the mutation at exon 8. Pseudodeficiency alleles were found in 9 (56%) of patients. The frequency of the poly A allele was 15.6%, and the glycosylation allele 12.5% among the patients' studied alleles. **Conclusion:** A great variability was observed in ASA enzyme activity in patients with MLD and their parents. The combination of pseudodeficiency alleles with MLD can complicate interpretation of the results in families at risk.

P0821. Molecular Screening of β -Thalassaemia Mutations in Iran

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We have characterized the β -globin gene mutations in 1800 carriers of β -thalassaemia and analyzed their regional distribution in Iran. The subjects were either the parents of affected thalassaemia patients or carrier couples identified following the national premarital preventive screening program. The majority of the carriers were from Mazandaran and Gilan in the Caspian Littoral in the North, Fars and Khuzistan in the South. Using the PCR-based allele-specific technique of ARMS the subjects were screened and the following alleles with 1% or greater frequency were detected in the following order: IVSII-1(G Δ A), IVSII-5(G Δ C), CD 36/37(-T), IVSII-1(G Δ A), CD 8/9(+G), CD 44(-C), IVSII-110(G Δ A), IVSII-25del, CD30(G Δ C), CD 8(-AA), CD 39(C Δ T), IVSII-745(C Δ G), IVSII-6(T Δ C). The frequency range was between 35.9% and 1% for the most frequent and least common mutations with almost 80% detection rate. The IVSII-1mutation was found in all population groups with varying frequencies. The other mutations also showed clustering with particular regions. To characterize the remaining 20%, the subjects were further investigated by DGGE and DNA sequencing. This approach has revealed 13 mutations so far. These are:

CD 37/38/39(-GACCCAG), CD22/24(-AAGTTGG), CD 22(G Δ T), CD 5(-CT), CD 25/26(+T), CD15(G Δ), CD 82/83(-G), IVSII-130, IVSII-850(G Δ T), -101(C Δ T), IVSII-128(T Δ G), -88(C Δ T) and the novel mutation of -26(A Δ C). This study shows that the underlying genetic determinants of β -thalassaemia in Iran is very heterogeneous. The generation of this data was crucial in setting up of the prenatal diagnosis in Iran.

P0822. A novel COX III deletion and D-Loop tandem duplications of the mitochondrial genome associated with rhabdomyolysis

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The mitochondrial DNA (mtDNA) is replicated without proofreading and efficient DNA repair mechanisms. With increased exposure to oxidative damage by reactive oxygen species and free radicals generated due to electron leakage of the respiratory chain, the DNA is subject to mutations. A vast number of point mutations and large-scale mtDNA rearrangements have been associated with a diversity of neuromuscular disorders and phenotypes. Rhabdomyolysis, the breakdown of myocytes resulting in the leakage of contents such as myoglobin into the blood and urine, has been associated with a number of mitochondrial DNA mutations including: tRNA^{Leu(UUR)} A3243G, G15059A, a 24 bp deletion in the cytochrome *b* gene, cytochrome *c* oxidase, COX I G5920A, COX II, a 15 bp deletion of COX III gene; and multiple mtDNA deletions resulting from nuclear defects. Short tandem duplications of the D-Loop region, the non-coding portion of mtDNA, have been associated with mutations in tRNAs or cytochrome *b* and along with large scale deletions are recognized as markers of early molecular events of the human aging process but have not been described in patient's younger than 30 years of age. Histochemical, immunohistochemical, genetic analysis of mtDNA and western blot analysis was carried out on a 27 year old patient with a single unprovoked episode of rhabdomyolysis. The muscle biopsy displayed prominent focal COX-negative muscle fibres and a novel one base pair COX III deletion causing a premature stop codon and was associated with multiple D-Loop duplications suggesting a combined deleterious effect.

P0823. Frequency of GJB2 deafness in Lur population of Iran and one novel GJB2 deafness-causing variant

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Mutations in the gene that encodes the gap-junction protein connexin 26, GJB2 gene, on chromosome 13q12 account for most autosomal recessive non-syndromic deafness (ARNSD) in many world population. Over 90 different ARNSD-causing mutations in GJB2 have been reported, but 35delG mutation is the most frequent one in different populations. In this study, we assessed the contributions made by GJB2 mutations to the ARNSD genetic load in Lur population of Iran. This population mainly lives in west of Iran. The study group consists of fifty three Lur probands suffering from ARNSD. The first step was an allele-specific polymerase chain reaction (ASPCR) assay to screen all study participants for the 35delG mutation. All samples excluding 35delG homozygotes were analyzed by DHPLC and sequencing. Seven GJB2 deafness-causing variants were found including: 35delG, W24X, 314del14, V95M, -3170G>A, 512insAACG and 510insCGAA. Among them 35delG mutation was the most frequent one and the 510insCGAA is a novel deafness-causing mutation which has not been detected in other world populations. In addition to the abovementioned mutations, the V153I polymorphism was detected in three patients. Taken together, nine patients (16.9%) had two GJB2 deafness-causing alleles. Based on our results, we suggest that other genes are the major responsible for ARNSD in this population.

P0824. MtDNA deletions in patient with hypertrophic cardiomyopathy-is there a causal relation?

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Within years 1995 - 2004, we have identified isolated or combined cytochrome *c* oxidase deficiency in 69 children (3 weeks till 16 years). Echocardiography revealed hypertrophic cardiomyopathy in 25 of them. This group of patients is intensively studied on the molecular level.

In proband with exercise intolerance, the hypertrophic cardiomyopathy was recognized at the age of 15 years. The results of histochemistry in endomyocardial biopsy (irregular decrease in COX activity) suggested the mitochondrial disorder caused by disturbance in mtDNA. Biochemical analysis in muscle biopsy of the patient revealed decreased activity and protein amount of complex IV and I. No mtDNA rearrangement was found in DNA isolated from muscle sample therefore mtDNA was sequenced and 2 mutations were found: 8348A>G and 12174C>T. Despite the 8348A>G mutation has been already associated with hypertrophic cardiomyopathy (Terasaki et al., 2001); we were not able to confirm the pathogenicity of both mutations. The mutations were homoplasmic in all analysed tissues of the patient and in blood of his 7 maternal relatives. Analysis of the heart muscle obtained at autopsy revealed the presence of mtDNA rearrangements. MtDNA deletions were found also in autopic muscle sample. These observations suggest worsening of the mtDNA quality during the course of the disease. To find the cause of the mtDNA deletions observed in our patient, the analysis of genes involved in a process of mtDNA replication is necessary. Supported by GAUK41/2004/c, GAUK153/2004/c and IGA MZ NR 8065/3.

P0825. Genetic studies of the Iranian deaf population

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During last six years, probands from 1254 families with hearing loss have been referred to our center for genetic testing. In 13 persons, the following syndromic phenotypes were recognized: Usher, Familial Expansile Osteolysis (FEO), Meniere, Bilateral Enlarged Vestibular Aqueduct (EVA), Waardenburg, Oto-Palato-Digital, Gorlin, Pendred, Stickler syndrome and Kallmann. In the remaining 1241 probands, the diagnosis of non-syndromic deafness was made. Pedigrees were consistent with autosomal recessive inheritance in 1163 probands who had consanguineous parents and other affected siblings, and autosomal dominant inheritance in ten persons. Three families segregated X-linked deafness. In 65 probands, no other affected relative could be identified - we classified these as simplex cases. GJB2 mutation screening was complete in 1094 patients with presumed autosomal recessive deafness, initially by completing an allele specific polymerase chain reaction (ASPCR) to detect the 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 deafness in 167 of 1094 familial cases (15.3%) and in 4 of 65 simplex cases (6.1%). Identified deafness-causing allele variants included: delE120, 167delT, R184P, 310del14, R32H, 314del14, 35delG, IVS1+1G>A, -3170G>A, R127H, W24X, R143W, E129K, 312del14, M93I, W77R, 8insT, 512insAACG, 510insCGAA, 507insAACG, 329delA, 363delC, Q80L. The last four are novel mutations, which have not been reported in other populations. Selected probands and families negative for deafness at the DFNB1 locus are beginning used to complete a genome-wide linkage analysis. In four of these families, the deafness-causing gene has been localized to specific chromosomal regions.

P0826. Detection of Cystic Fibrosis mutations in echogenic bowel

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The presence of echogenic bowel detected on ultrasound investigation during the second trimester of pregnancy can be a normal variant and also associated with cystic fibrosis, as well as a number of other disorders such as chromosome aneuploidy. We have investigated 91 cases of echogenic bowel and have detected cystic fibrosis homozygosity in four of these (4.3%) with the following genotypes: F508del/L732X, F508del/574delA, F508del/F508del, F508del/G542X. In addition heterozygosity was observed in 15 cases (13.4%): 9 with F508del 2 with 621+1G>T and 1 case each for I148T, 2789+5G>A, R297Q and E822X. An additional finding was the presence of particular polymorphisms: 3 instances of 1716G/A (E528E), 2 of 2752-15G/C, 2 of R1162L and 1 of 4029A/G. Our data indicated that the prior risk of CF in a fetus with echogenic bowel for our population is 4.3% and the remaining risk of an heterozygous fetus having CF is between 10.3 and 20%.

Table 1: Summary of families tested and CF results

Both parents and/or fetus negative	21	
One parent carrier-fetus negative	2	I148T, F508del
One parent carrier-fetus not tested	5	621+3A>G, R75Q and 3 with F508del,
One parent carrier-fetus carrier	7	621+1G>T, I148T and 5 with F508del
Both parents carriers- fetus affected	4	F508del/L732X, F508del/574delA, F508del/F508del, F508del/G542X
Fetus only tested-negative	18	
Fetus only tested-carrier	8	E822X; 2789+5G>A; 621+1G>T; R297Q; and 4 F508del
Couple only tested-both negative	26	
TOTAL	91	4 homozygotes and 15 heterozygotes

P0827. Two novel CFTR gene mutations identified in cystic fibrosis patients from Lithuania: can the 4171insCCTA mutation be associated with low sweat chloride level?

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The spectrum and frequencies of CFTR gene mutations in cystic fibrosis (CF) patients from Lithuania were investigated in the present study. CF patients were initially tested for F508del using PCR with oligonucleotide primers bridging the deletion. Subsequently they were screened for the presence of other mutations in all CFTR gene exons using DGGE and sequencing. Duplex PCR was applied to identify CFTRdelle2,3 (21 kb). 47 patients with CF residing in Lithuania (94 CF chromosomes) were investigated.

Twelve different CFTR gene mutations were identified: F508del (70.21%), R553X (4.26%), N1303K (4.26%), CFTRdelle2,3 (3.19%), and single cases (1.06%) of W1282X, G314R, R1066H, 3667insTCAA, 574delA, 4006-4A>G, 794delC, and 4171insCCTA. Mutations on 9 CF chromosomes (9.6%) were not identified.

Frameshift mutations 794delC and 4171insCCTA have not been described previously. Both patients having CFTR genotypes [F508del]+[794delC] and [F508del]+[4171insCCTA] had a moderate CF clinic with pancreatic insufficiency and pulmonary diseases. Sweat chloride concentration was estimated for these patients. Chloride concentration for a boy having [F508del]+[794delC] genotype was 107 mmol/L. Sweat chloride test was repeated fivefold for 1 year old boy with [F508del]+[4171insCCTA] genotype, the estimated concentration was unusually low 4-9 mmol/L.

In conclusion the applied PCR-based approach resulted in 12 different CFTR gene mutations in CF patients from Lithuania with the overall identification rate of 90.4%. 4171insCCTA and 794delC mutations were novel. The patient having genotype [F508del]+[4171insCCTA] had unusually low sweat chloride level.

P0828. Good news for potential DMD/BMD carriers - MLPA gives the answer

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Duchenne and Becker muscular dystrophies (DMD/BMD) are common X-linked disorders with an incidence of 1 in 3500 live male births. Up to 1/3 of the cases are caused by de novo mutation, without any family history. About 60% of DMD patients have a deletion of at least one out of 79 exons of the large dystrophin gene. The identification of female carriers has been difficult, time consuming and not fully reliable till now. Using multiplex ligation-dependent probe amplification (MLPA) we reinvestigated 14 women from 6 families with an isolated case of DMD. In two families the carrier status of three women at risk was confirmed. Previously performed haplotype analysis was neither able to confirm nor to exclude the risk of these women being carriers of the deletion. Besides, using MLPA we examined 5 suspected patients without a deletion, however we didn't reveal any. We confirmed a deletion in one exon with three patients (exon 43, 44 and 51, respectively). With one patient the deletion of exon 19 was found to cover exons 18 to 30, in fact.

We show an interesting case of a female proband with DMD manifestation, who has a deletion of exons 46-51. This deletion was inherited from her mother and was not passed on her children.

We find MLPA to be a helpful and sensitive method of uncovering the carrier status in families with a deletion in DMD gene.

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P0829. Molecular detection of nondeletional mutations in alpha thalassemic patients by a DHPLC based assay

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Alpha-Thalassemias (α -Thal) are inherited disorders of hemoglobin synthesis that arise from more than 80 different genetic alterations within the α -globin gene cluster in 16p13.3. The great majority of pathogenic mutations consist in large genomic deletions that involve either one or both α -globin genes ($\alpha 1$ and $\alpha 2$), while nondeletional mutations (including point mutations and deletions or insertions of few nucleotides) are responsible for only a minority of α -thalassemia phenotypes.

We have developed a rapid and reliable method for the molecular detection of α -globin nondeletional defects using a denaturing high-performance liquid chromatography (DHPLC)-based assay. Subsequently, we have analyzed 70 Italian subjects, who resulted negative after a previous screening for the most common α -globin large genomic rearrangements, using our DHPLC method. The full genomic region of both α -globin genes were carefully amplified in four overlapping fragments and then subjected to DHPLC analysis. Overall, using the DHPLC-based method we have unambiguously identified both common and rare mutations, as well as neutral variants. The rapidity and reliability of this approach seem appropriate for a second-level molecular diagnosis of α -thalassemia carriers and/or patients that are negative for the most common deletional mutations.

P0830. Three novel mutations in CX32 gene detected by DHPLC mutation analysis

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X-linked dominant Charcot-Marie-Tooth disease (CMTX) is an inherited neuropathy caused by mutations affecting the gap junction protein beta

1 (GJB1) gene coding for the gap junction protein beta 1 (connexin32) located in the X-q13 region. In males symptoms begin in late childhood or adolescence and progress to moderate disability by the third decade of life. Female carriers are usually less severely affected than males at the same age. Over 250 different mutations in the GJB1 gene have been identified involving all portions of the Cx32 protein.

We propose a mutational approach for the Cx32 gene based on denaturing high performance liquid chromatography (DHPLC), a very fast and sensitive method that minimizes diagnosis time and cost. We studied seven unrelated Italian cases diagnosed as probable CMT based on typical clinical features of distal wasting, hyporeflexia with distal sensory disturbance. In six patients a positive family history was present. We detected by DHPLC three novel (Ser49Phe, Ser128Leu, and Phe153Leu) and four known mutations (Tyr7Cys, Arg164Gln, Arg183Hys, Arg183Cys). All mutations segregated with the disease in the family, except the Ser49Phe mutation that is a de novo mutation. The three novel substitutions were yet unknown, but there were other amino acid changes reported at those amino acid positions. Both S49 and Phe153 occur in the extracellular domain of the Cx32, instead the Ser128Leu occurs in the intracellular domain; however all three mutations are also highly conserved in the GJB1 proteins among all mammalian species suggesting a functional role for the aminoacid at these positions.

P0831. Molecular analysis of candidate genes of LQT syndrome

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Understanding the molecular basis of cardiac channel activity has been of major interest in recent years. The Long QT syndrome (LQTS) is an inherited cardiac disorder in which ventricular tachyarrhythmias predispose affected individuals to syncope, seizures, and sudden death. Ventricular repolarization involves several distinct currents controlled by a number of different ion channels. The defect in any of these channel genes can result in altered repolarization leading to LQTS. Presently six different genetic loci are associated with LQTS: KCNQ1(11p15.5), KCNH2(7q35-36), SCN5A(3p21-24), Ankyrin-B(4q25-27), KCNE1(21q22.1-22.2) and KCNE2(21q22.1). Mutations in some other genes appear to be associated with LQTsyndrom. The candidate genes are SCN1B (β -subunit of I_{Na}), KCND3 (α -subunits of Kv4.3 of I_{to1}), CACNA1C (α -subunit of I_{cal}) and KCNJ2 gene (α -subunit of I_{K1}).

We used single strand conformational polymorphism (SSCP) analysis to screen all exons of KCNQ1, KCNH2, KCNE1 genes and recently of SCN1B gene in group of unrelated Czech LQT patients. SSCP analyses were followed by sequence analyses of aberrant conformers.

DNA sequence analysis determined one frameshift mutation and seven missense mutations of KCNQ1 gene, four missense mutations of KCNH2. Some of them were novel. We also identified nine different single-nucleotide polymorphisms (SNPs) in KCNQ1 and KCNH2 and three variants of introns in KCNH2 gene. The screening for mutations in the KCNE1 gene revealed rare amino acid variants G38S and D85N. However, functional studies of these determined sequence variants would be required to absolutely confirm the pathogenic nature of these changes

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P0832. One Usher case, two genes, three mutations.

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Usher syndrome type I (USH1) is manifested by profound congenital deafness, vestibular dysfunction and retinal degeneration beginning in childhood. Five USH1 genes have been identified (MYO7A, CDH23, USH1C, PCDH15, SANS). The proband is a twelve years old female, single child with non affected parents. At three months old, nystagmus was noticed and ophthalmic examination confirmed the diagnosis of retinitis pigmentosa (RP). The age of walking was delayed until 18 months due to vestibular dysfunction. When she was

3 years old, profound deafness was diagnosed and cochlear implant surgery was performed. Molecular studies in the CDH23 identified two novel deleterious mutations. Segregation analysis showed that the two mutations were on different chromosomes and thus the proband presents the [c.6146_6153delTCAACAGC]+[IVS7-2A>C] genotype. Moreover, for epidemiologic purpose, systematic screening for the 2299delG mutation in the USH2A was performed. The child carries in addition to the CDH23 genotype the 2299delG mutation that was inherited from her mother. This mutation is common in Usher type 2 (moderate-to-severe deafness) but has also been described in autosomal recessive non-syndromic RP.

Recently, digenic inheritance of USH1 phenotype in families with mutations in CDH23 and PCDH15 genes has been shown. This case study supports further that USH1 and USH2 arise from distinct pathogenic processes since no digenic effect is being observed in the mother who is double heterozygote for a CDH23 and USH2A mutation. However, the association of these three deleterious mutations localized in two Usher genes is likely to explain the severe RP phenotype described in this proband.

P0833. Characterization of Ozzy, a mouse model for Alagille syndrome

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Ozzy originated from an ENU-mutagenesis program and was selected because of its headbobbing phenotype. We examined the vestibulo-ocular reflex (VOR) and the optokinetic reflex. Ozzy mice showed no VOR, indicating severe functional defects in the semicircular canals and ampullae. CT-scanning of the inner ears confirmed these results showing narrowing and truncations of at least one of the semicircular canals and loss of the ampullae. Hearing abilities were tested using frequency-specific Auditory-evoked Brainstem Response, revealing a slight threshold increase for the middle frequencies. Linkage analysis localised the gene in an 8.7 cM region on chromosome 2, and a 499 T->A missense mutation was identified in Jag1, leading to a substitution of an evolutionary conserved tryptophane (Trp167Arg). Mutations in the human homologue of Jag1 cause Alagille syndrome (AGS), an autosomal dominant disorder associated with liver, heart, eye and skeletal abnormalities, accompanied by a characteristic facies. Occasionally it affects other organ systems like the kidney or the hearing apparatus. Liver disease is the main diagnostic factor for AGS. Ozzy mice showed significantly less interhepatic bile ducts than wild-type littermates ($p=0.011$). 20% of Ozzy mice showed dilation of the right ventricle in combination with an opening in the tricuspidal valve and aortic dextroposition, symptoms of the tetralogy of Fallot, the most common heart defect in AGS. No eye or vertebral abnormalities could be detected. Since Ozzy displays two of the major and one minor characteristic of AGS, we conclude that this mouse can be considered to be an animal model for Alagille syndrome.

P0834. Abnormal inheritance and phenotypic consequences of the A1555G mutation in a heteroplasmic family affected of nonsyndromic hearing loss

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Most mitochondrial pathogenic mutations appear in heteroplasmy, being lethal in the homoplasmic state. However, the A1555G mutation in the mitochondrial 12S rRNA gene, which has been associated with aminoglycoside-induced and nonsyndromic hearing loss, appears mostly in homoplasmy. Moreover, different clinical phenotypes have been associated with this mutation even in the absence of aminoglycoside exposure. We report here the identification of a Spanish family with an abnormal inheritance pattern of the A1555G mutation in heteroplasmy. Mutation load of all available members have

been quantified using the pyrosequencing technology. As a result, we have observed a great variability in the mutation load of heteroplasmic A1555G carriers. The mutation load in the offspring of heteroplasmic mothers showed a tendency to reverse to homoplasmy after three generations. The study of genotype-phenotype suggests that there is a threshold in mutation load for manifestation of clinical symptoms. It is well known that in the case of heteroplasmy, different proportions of wild-type and mutant mtDNA accumulate in different tissues. Thus, we should consider that the estimation of mutation load obtained from peripheral blood might not reflect the real situation of heteroplasmy in the inner ear, and speculate that unaffected subjects that have the mutation have a high proportion of the normal 12S rRNA gene. In summary, this family provides evidence of the presence of a certain amount of wild-type mtDNA in subjects with the A1555G mutation, which could act as an additional modifying factor of the hearing phenotype in subjects carrying the mutation which show little hearing abnormalities.

P0835. Molecular analysis of the porphobilinogen deaminase gene in newly (2004) diagnosed Czech and Slovak acute intermittent porphyria patients: Report of three novel mutations

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Background: Acute intermittent porphyria (AIP) is autosomal dominant disorder caused by the partial deficiency of porphobilinogen deaminase (PBGD; or hydroxymethylbilane synthase, HMBS; EC 4.3.1.8), the third enzyme in the heme biosynthetic pathway. It is manifested by life-threatening acute neurological attacks that can be provoked by various exogenous factors. Clinical expression is highly variable, and ~90% of AIP heterozygotes remain asymptomatic throughout life. To date, over 250 PBGD mutations have been identified.

Objective: To identify the molecular lesions in newly diagnosed (2004) Czech and Slovak AIP patients.

Design and methods: Genomic DNA was isolated from members of seven unrelated AIP families from Czech and Slovak Republics, and mutation screening was performed by PCR and denaturing gradient gel electrophoresis (DGGE). Subsequently, automated DNA sequencing was used to verify the mutated lesions. For each identified mutations, a restriction fragment length polymorphism (RFLP) assay was established, and a total of 36 individuals from seven families were analyzed to detect asymptomatic carriers.

Results: Eight mutations were identified, including three novel mutations (610 C>A, 675 delA, 966 insA), and five previously reported mutations (76 C>T, 77 G>A, 518 G>A, 771+1 G>T, 973 insG). This is the first report of the 518 G>A mutation in the Czech and Slovak population.

Conclusions: Three novel mutations were identified in seven unrelated AIP families. These studies further emphasize the molecular heterogeneity of AIP, and provide accurate detection of asymptomatic carriers.

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P0836. Molecular characterization of hereditary spastic paraparesis (HSP) in Israel

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Background. Hereditary spastic paraparesis (HSP) is a clinically and genetically heterogeneous group of pure and complex forms with various genetic loci corresponding to autosomal dominant (AD) and autosomal recessive (AR) forms. The spectrum and frequency of HSP forms in Israel are currently unknown, although instances of unique and important syndromes have been reported.

Objective. To set up a clinical database and perform molecular characterization of HSP in Israel

Methods. 15 unrelated multiplex Israeli families with HSP formed the basis of the present study. The mode of inheritance was apparently AD

in 9 families and AR in 6. Clinical diagnosis and patient ascertainment was established by the accepted criteria. Genotyping was performed on genomic DNA using PCR amplified polymorphic markers for the 3 most common AD (*SPG4*, *SPG3A*) and AR (*SPG11*) loci and fluorescein-labeled primers.

Results. As expected, AD-HSP mainly presented the pure clinical phenotype whereas AR-HSP patients manifested additional neurologic manifestations, such as mental retardation, peripheral neuropathy and thin corpus callosum observed in 3 families. Of the 10 informative families, 2 showed linkage to the *SPG4* locus, 2 to the *SPG3A*, and 2 to the *SPG11*. Mutational screening and studies with additional markers are in progress.

Conclusions. Despite the apparent referral bias, the observed proportion of AR-HSP forms seems important and may be related to the common preference of parental consanguinity. Molecular characterization of HSP is expected to enable better dissection of the various clinical syndromes allowing genotype-phenotype correlation and accurate genetic counseling to family members.

P0837. Increased Brain-Derived Neurotrophic Factor (BDNF) plasma levels in eating disorders: a discordant sib-pair analysis

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Murine model approaches and association studies have indicated that the Brain-derived neurotrophic factor (BDNF) gene participates in the susceptibility to eating disorders (ED). The association of the BDNF gene with anorexia nervosa (AN) and bulimia nervosa (BN) and the generation Bdnf knock-out mice support that increased levels of BDNF in the central nervous system (CNS) could be involved in restricting food intake and in low body weight. Thus, we propose that ED are characterized by alterations in blood BDNF levels as a reflection of increased BDNF concentration in several regions of the CNS. To test this hypothesis we assessed the BDNF plasma levels in 50 discordant sib pairs with ED by using an Enzyme Linked Immunoassay System (BDNF Emax Immunassay System, Promega) and found that BDNF levels were significantly higher in ED patients than in their unaffected sibs (mean 57.7 ng/ml vs mean 40.9 ng/ml; P = 0.004). No significant differences were found between the different ED categories (AN and BN) or considering the purging and non-purging ED phenotypes. We also observed a negative correlation between Body Mass Index (BMI) and BDNF plasma levels (P = 0.015). These results give further physiological evidence for a role of this neurotrophic factor in AN and BN, and strongly argue for its involvement in eating behavior and body weight regulation.

P0838. The MEFV L110P-E148Q complex allele associated with an unusual presentation of familial Mediterranean fever

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Familial Mediterranean Fever (FMF) is caused by mutations in the MEFV gene. FMF presents typically as recurrent episodes of fever and polyserositis; amyloidosis is an important long-term complication. Genetic analysis is valuable in confirming the diagnosis and assisting therapeutic decisions.

We have tested 273 individuals by sequencing exons 2 and 10, followed by complete gene sequencing when appropriate, identifying one or two mutations in 93 (34%). Eleven distinct mutations have been found, most commonly M694V (51%), M680I (19%) and V726A (8%). 12% of patients were hetero- or homozygous for E148Q, which was initially considered as a low-penetrance mutation but which is now being "re-evaluated" as a non-pathogenic polymorphism.

We describe a 23-year old Turkish woman with a short history of "sore throat", fever, and abdominal pain that resulted in explorative laparoscopy. Following the intervention severe SIRS with rash,

arthralgia, and polyserositis with pleural effusions and ascites developed. After exclusion of infections and other rheumatic/ autoimmune diseases, genetic diagnosis of FMF was requested and the patient identified as homozygous for both L110P and E148Q. Colchicine treatment was initiated, and the patient recovered.

L110P has been previously reported in just 3 patients; in at least two, it was present in a complex allele with E148Q. The overall frequency of L110P cannot be reliably assessed at present, as many laboratories do not include it in their testing. We predict that L110P will be commonly found in cis with E148Q, and that attention is required to avoid falsely identifying heterozygotes as having "two mutant alleles".

P0839. Functional analysis of a new human *RUNX2* mutation found in a family with cleidocranial dysplasia

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Mutations of *RUNX2* gene results in cleidocranial dysplasia (CCD), which is a dominantly inherited skeletal dysplasia characterized by hypoplastic clavicles, large fontanelles, dental anomalies and delayed skeletal development. *RUNX2* encodes for an osteoblast-specific transcription factor, which recognizes specific DNA sequences by the runt domain. Most of missense mutations described in CCD patients affect conserved residues of the runt domain, abolishing both the DNA-binding and transactivation functions and produce a classic CCD phenotype. We have recently found a novel c.574G>A *RUNX2* missense mutation present in affected members of a family with clinical diagnosis of classical CCD. This mutation causes the change of the glycine at position 192 in arginine (G192R), in the runt domain. A previous study describing mutations of neighbouring residues 190, 191 and 193 showed that missense mutations affecting these positions produced complete loss of both DNA-binding activity and in vitro transactivation. In this study, the molecular properties of this mutation have been investigated. In gel-retardation assay experiments, the DNA-binding activity of a bacterially-expressed runt domain bearing the G192R mutation is mildly reduced with respect to the wild-type protein. However, in cell transfection studies, using the osteocalcin as target promoter, the mutation completely abolished the activating properties of the protein. These results suggest that G192R mutation effect is only in part explained by impairment in DNA binding activity.

P0840. Pathogenic mutation (R1329H) or normal variant in Tuberous Sclerosis?

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The diagnosis of Tuberous Sclerosis Complex (TSC) is based on clinical findings. Two causative genes, *TSC1* and *TSC2*, have been identified. Missense mutations are common in *TSC2*, and the difficulties in interpreting the significance of this type of mutation are illustrated by a case referred to our molecular study of 65 TSC patients.

TSC1 and *TSC2* mutation screening of a 4 y old boy with congenital astrocytoma identified a mutation, c.3986G>A; p.R1329H, in *TSC2*. This missense mutation has previously been reported in a TSC patient and considered putatively pathogenic. However, the mutation was also found in our patient's clinically normal mother. Hence, the R1329H mutation is more likely to be a normal variant.

Clinical re-evaluation of our patient revealed that the astrocytoma was the anaplastic type and not the giant cell type, typically seen in TSC. Cerebral MRI did not show cortical tubera or subependymal glial noduli. Renal ultrasonography, echocardiography, eye and skin examination were normal. Conclusively, the patient did not fulfil the diagnostic criteria for TSC.

Intriguingly, the functional studies of the corresponding tuberin mutant indicated modestly impaired growth repressing function. It can therefore be speculated that this missense mutation can give rise to abnormal cellular growth in limited tissues or a TSC phenotype depending on the genetic background.

P0841. Results of molecular-genetic analyses in the group of probands with the Prader-Willi and Angelman syndromes

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The Prader-Willi and Angelman syndromes are two distinct multisystem disorders in the chromosomal region 15q11-q13, the genetic background of these disorders is complex and they are caused by absence of expression of the paternal (PWS) and maternal (AS) active genes.

We analyzed 99 PWS and 86 AS suspected probands in our study. We applied following methods for investigation: microsatellite analysis (loci: LS6-CA (D15S113), GABRB3 and IR4-3R (D15S11)) with all patients, methylation analysis with 80 PWS or AS patients and FISH analysis with UPD suspected cases.

We identified 29 of 99 (29 %) positive PWS probands with the following distribution: 20 deletions (69 %), 6 uniparental maternal heterodisomies (21 %), 2 uniparental maternal isodisomies (7 %). In 1 case, the absence of paternal 15q11-q13 region was excluded by STR analysis, but methylation analysis showed inadequate methylation pattern, identifying the imprinting mutation (3 %).

We detected 20 of 86 (23 %) positive AS probands with the following distribution: 18 (90 %) deletion forms, 1 (5 %) uniparental paternal isodisomy and 1 trisomy of chromosome 15 (5 %). Besides, we detected a recombination between loci LS6-CA (D15S113) and GABRB3 in 1 suspected case with AS. This case is still being analyzed.

Our results agree with other published data. Complex diagnostic approach allows reliable genetic prognosis for the patients.

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P0842. Genetic analysis of *SLC12A3* gene of patients with Gitelman syndrome in the Czech Republic

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To investigate the causes of inherited hypokalemic metabolic alkalosis associated with Gitelman syndrome, we have been searching for mutations in the *SLC12A3* gene (thiazide-sensitive NaCl cotransporter). This inherited autosomal recessive renal disorder is linked with hypokalemic alkalosis, significant hypomagnesemia and low urinary calcium, and is manifested usually after the age of six. DNA was isolated, amplified and analysed for mutation detection with SSCP and subsequent sequencing. 16 patients with characteristic clinical features - abnormal levels of specific ions in blood and urine (Mg^{2+} , K^+ , Ca^{2+} ...) - have been collected from different regions of the Czech Republic.

Genetic analysis revealed several novel mutations. One of the patients, a 30-year-old man, manifested in childhood hypokalemia and paresthesias of the upper extremities. He had a mild degree of mental retardation. Our laboratory tests confirmed hypokalemia (2.2mmol/l), metabolic alkalosis (pH of blood 7.54, HCO_3 37mmol/l), hypomagnesemia (0.41mmol/l) and hypochloremia (91mmol/l). The urinary excretion of calcium was decreased (2.33 mmol/24h). We also noted increased levels of plasma renin activity (PRA) and tubular dysfunction. Genetic analysis revealed the mutation c.1315G>A (p.Gly439Ser).

Among other mutations found are c.1222A>C (p.Thr408Pro) and c.480dupC (p.Pro160fsX97). Mutation detection is still in progress for all of the 16 patients.

P0843. A skewed X-inactivation mechanism could explain the severe hemophilia B feature of a 3-year old girl with a severe hemorrhagic diathesis.

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Hemophilia B is a X-linked inherited hemorrhagic disease resulting from deficiencies of blood coagulation factor IX. Usually hemophilia is transmitted by healthy heterozygous female carriers to affected males. Very rarely can a woman exhibit hemophilia B and the most common explanations for how this can happen include compound heterozygosity and skewed X-inactivation. Few, isolated case-reports exist, so that we ignore the frequency of hemophilia B in women and whether the clinical phenotype is the same of that encountered in males. Our experience regards a 3 year-old little girl suffering from a severe hemorrhagic diathesis. The first bleeding was an apparently spontaneous hemarthrosis of the left knee, afterward she presented a large hematoma of left ilio-psos muscle. Laboratory data showed a prolonged PTT (R=1.97, n.v. 0.9-

1.2) and the dosage of F.IX <1% (n.v. 60-140%). No underlying diseases were found, an acquired inhibitor was excluded. The final diagnosis was strictly ascribed to inherited severe hemophilia B. In both the episodes the patient needed substitutive treatment with recombinant F. IX concentrate, reaching the complete resolution of bleedings with a good clinical outcome. The sequence of the entire coding region of the F.IX gene and the exon-intron boundaries was done. We have identified a heterozygous C-to-T transition within the exon 6 leading to an Arg-to-Trp substitution at position 180. This mutation, described in several hemophiliacs B, was inherited from her mother. Since no further mutation was identified, we suggest that a skewed X-inactivation mechanism could be responsible for the disease.

P0844. Novel spastin mutations in two Italian patients with hereditary spastic paraparesis

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Hereditary spastic paraparesias (HSP) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive spasticity and hyperreflexia of the lower limbs. Autosomal dominant hereditary spastic paraparesis linked to chromosome 2p (SPG4) is the most common form of HSP. It is caused by mutations in the SPG4 gene encoding spastin, a member of the AAA protein family of ATPases. Over 100 spastin mutations including missense, nonsense and splice-site point mutations, as well as little insertions and deletions have been described. A mutational screening of spastin gene by Denaturing High Performance Liquid Chromatography (DHPLC) on Italian patients diagnosed as probable HSP revealed two novel mutations in exon10: a missense mutation C1429T and a one base pair insertion (1406insT). The C1429T missense mutation replaces a proline at position 435 with leucine (P435L) and was not found in 100 normal chromosomes. The one base pair insertion (1406insT) leads to frame shift (aa 427-441) and premature termination (442stop codon); it produces a truncated spastin protein lacking the ATPase domain, the AAA cassette region, and one of two leucine zipper domain. Both mutations could impair the spastin function through either a dominant negative effect on the wild-type spastin or by a loss of function of the mutated protein.

P0845. Molecular studies of ORF15-RPGR in French patients: advantages of the PTT test approach.

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Among the various forms of inheritance, X-linked RP (XLRP) represents the severe form with an early age of onset of the disease and rapid progression. Of the five loci mapped on the X chromosome,

retinitis pigmentosa GTPase regulator (RPGR), represents the major gene as it accounts for 70-90% of XLRP. The majority of the mutations is clustered in an alternative 3' terminal exon, ORF15, that represents the major splice variant of the transcript. Molecular studies of ORF15 is known to be technically challenging because of its highly repetitive sequence. To avoid systematic difficult sequencing and since all the mutations described in this exon lead to premature termination of translation, we have investigated the efficiency of the Protein Truncation Test (PTT) as a scanning method.

The size (1,7Kb) of the ORF15 exon allows working on genomic DNA directly. After in vitro transcription and translation, the translated proteins are separated by SDS-PAGE and autoradiography is performed. The existence of abnormal pattern migration, compared to a wildtype peptide, identifies a translation terminating protein. ORF15 exon sequencing is performed with multiple internal primers to confirm the presence of a mutation.

We show that a PTT pattern is specific of a premature translation termination as in our cohort we found that 10 different mutations, leading to 6 different premature STOP codon, are associated to 6 different PTT pattern. Because a PTT pattern is so far specific of a premature STOP codon, therefore one can limit the sequencing effort to a restricted portion of the exon.

P0846. Molecular diagnosis of Charcot-Marie-Tooth disease : strategy refinement and results of the genetic exploration of a series of 200 patients in Marseille.

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Hereditary sensitive and motor peripheral neuropathies also called Charcot-Marie-Tooth disease (CMT) constitute a frequent demand for genetic testing. This group of pathologies is characterised by its genetic heterogeneity. All modes of inheritance have been reported, and to date more than 20 genes have been identified as mutated in CMT, while some others are still only localized, making the classification complex and constantly evolving. While electrophysiological criteria only allow distinction between demyelinating (CMT1), axonal (CMT2) or intermediate neuropathies, clinical history can sometimes orientate the molecular explorations but rarely allows focusing on one particular gene. Furthermore, there is now evidence that several of the identified genes are involved in axonal as well as in demyelinating forms (e.g. *P0*), and/or in dominant as well as recessive CMT (e.g. *P0*, *EGR2*). In this context, defining a molecular strategy for genetic testing has become a real challenge that our laboratory is facing since 1997. According to recent data we explore at present 10 different genes of CMT: *PMP22*, *P0*, *LITAF*, *EGR2*, *GJB1*, *MTMR2*, *GDAP1*, *NDRG1*, *MFN2* and *LMNA*. Among these genes, some are explored in particular ethnic background situations (e.g. *NDRG1*) and/or in case of associated evocative clinical features (e.g. *LMNA*). The mutation frequency of newly identified genes (e.g. *MFN2*) remains to be determined. We wish to present here our strategies in molecular exploration of CMT patients, and the results obtained on 200 patients included in such a genetic testing in our laboratory in Marseille.

P0847. Mitochondrial content reflects oocyte variability and fertilization outcome

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OBJECTIVE: To determine the content of mitochondrial DNA (mtDNA) in oocytes from a range of patients with fertilization success and failure. **DESIGN:** Analysis of the mtDNA content in fertilized and unfertilized oocytes and embryos using real time PCR. **SETTING:** University hospital infertility and research center. **PATIENT(S):** Fifty-four women seeking infertility treatment. **INTERVENTIONS:** None. **MAIN OUTCOME MEASURES:** 142 fertilized and unfertilized oocytes were classified into three main groups. Group I consisted of 35

fertilized oocytes from 21 patients; Group II 65 unfertilized oocytes from 36 patients; and Group III 42 degenerate oocytes from 23 patients. MtDNA content was determined by SYBR Green real time PCR based assay. RESULTS: The mean mtDNA copy number for the fertilized oocytes was 250,454 whilst for the unfertilized group this was 163,698 ($p<0.002$). There were significant differences for mtDNA copy number between the male factor and female factor infertility unfertilized oocytes ($p<0.02$) and for the unexplained infertility with female factor infertility groups ($p<0.005$). The mean copy number for the degenerate oocyte group was 44,629, which was significantly different to the other subdivisions in this group. CONCLUSIONS: It is evident that mtDNA content is critical to fertilization outcome and serves as an important marker of oocyte quality explaining some cases of fertilization failure.

P0848. PDS gene (SLC26A4) is frequently mutated in non syndromic hearing loss with inner ear malformation DFNB4.

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Sensorineural hearing loss HL is the most frequent sensory deficit of childhood, and is of genetic origin in up to 75% of cases. It has been shown that mutations of the *SLC26A4* (*PDS*) gene were involved either in syndromic deafness characterized by congenital sensorineural hearing loss and goitre (Pendred's syndrome), as well as in congenital isolated deafness (DFNB4). While the prevalence of *SLC26A4* mutations in Pendred's syndrome is clearly established, it remains to be studied in DFNB4. In this report, 112 patients from 103 unrelated families, with NSHL and inner ear malformation, were genotyped for *SLC26A4* using DHPLC molecular screening and sequencing. Eighty-eight allelic mutations were observed in 103 unrelated families, of which eighteen have never been reported. Most mutations were found in exons 6, 10, 16, 19 and 14, and many of these have already been described in Pendred's syndrome. The prevalence of *SLC26A4* mutations was 36.9% (38/103), with compound heterozygosity in 19.4% (20/103), while one patient was homozygous. All patients included in this series had documented deafness (age of onset, severity, type, evolution status), associated with inner ear malformation (enlarged vestibular aqueduct, cochlear dysplasia) and without any evidence of syndromic disease. Among patients with *SLC26A4* mutations, deafness was more severe and fluctuated more than in patients with no mutation ($p<0.05$). In conclusion, the incidence of *SLC26A4* mutations is high in patients with isolated deafness and inner ear malformation and could represent up to 6% of NSHL. Therefore, deaf children with inner ear malformation should undergo *SLC26A4* molecular screening.

P0849. Delineation of the candidate region for mental retardation in subtelomeric 1q deletions

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Subtelomeric deletions of chromosome 1q have been reported in several screening studies of mentally handicapped. We identified a patient with a de novo subtelomeric 1q deletion of approximately 8Mb.

To increase our insights in the mechanisms responsible for these subtelomeric rearrangements, we are trying to identify the breakpoint sequences of our subtelomeric deletions. Currently, the breakpoint region for our 1q patient was refined to a 18 Kb XmnI fragment close to the RGS7 gene. The RGS7 gene, a regulator of G protein signaling, seemed a strong candidate gene for the mental handicap as this neuron specific gene is primarily expressed in brain tissue and plays

a putative role in synaptic vesicle exocytosis. However, patients in two separate families have been reported with an interstitial deletion taking away RGS7 and at least five other genes without developmental delay or even mild dysmorphic features. This suggests that genes in the most distal 6 Mb to the telomere are associated with mental retardation. The most distal 1.5 Mb contain predominantly putative genes and/or pseudogenes resembling olfactory receptors, characteristic for many other chromosome ends. Thus, the genes between the olfactory receptor cluster and the deletion may be responsible for the clinical phenotype. This region spanning 4.5 Mb, contains 17 known genes including 5 zinc fingers: ZNF496, ZNF124, SBZF3, SMYD3 and ZNF238. Interestingly, related zinc finger genes have been implicated in other mental retardation disorders.

P0850. BBS4 interacts with dyactin 1 in a manner compatible with its intracellular transport by the dynein/dyactin complex

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Dyactin 1, in concert with dynein and other complexed proteins, e.g. dyanamin, mediates retrograde transport of intracellular protein cargoes along the microtubule cytoskeleton. We have shown previously that wild-type BBS4, the protein product of a gene mutated in Bardet-Biedl syndrome, interacts with full-length dyactin 1. In this study, dyactin 1 deletion constructs were made and their interaction with BBS4 was examined using both immunoprecipitation and cellular localisation studies. By immunoprecipitation, it was found that BBS4 interacts with the C-terminal portion, amino acids 879-1278, of dyactin 1. Deletion constructs of dyactin 1 consisting only of amino acid residues from within this region formed discrete spherical accumulations when transfected into 3T3 cells. BBS4-GFP, usually centrosomally localised, became aberrantly localised in the presence of the C-terminal dyactin deletion constructs, often colocalising with the cytoplasmic accumulations of the truncated dyactin 1. The previously characterised cargo-binding region of dyactin 1 to which BBS4 binds is distinct from the microtubule (amino acids 1-150) and dynein intermediate chain (amino acids 100-811) binding domains. The results shown here indicate that BBS4 binding of dyactin 1 is compatible with its transport by the dyactin/dynein complex. These findings support the previously discussed putative role of BBS4 as an adaptor protein for the transport of other centrosomally destined cargoes, such as pericentriolar material 1.

P0851. A Wilson disease patient homozygous for the Met645Arg mutation. Genotype-phenotype correlations.

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Wilson disease is an autosomal recessive disorder affecting copper transport, resulting in intracellular copper accumulation that produces hepatic and/or neurological damage. Molecular analyses of the ATP7B gene in Wilson disease patients have identified more than 200 different alterations. Our previous results in molecular diagnosis of Spanish patients showed a high allelic heterogeneity, what makes difficult genotype-phenotype correlations in our population. We have now identified a patient homozygous for the Met645Arg mutation. Despite the high frequency of this particular alteration in the Spanish population, this is the first carrier of Met645Arg in both alleles. The patient was casually diagnosed following a routine clinical screening when he was 52 years old. Minimal alterations in the hepatic biochemical values led to additional analyses. Low serum ceruloplasmine, low serum copper levels and raised copper urinary elimination after penicillamine were found, suggesting a possible Wilson disease. SSCP analysis of the ATP7B gene was performed, and sequencing of the shifted exons revealed a T1934G substitution (exon 6) in both alleles. Considering the low clinical affection of this patient, we suggest that Met645Arg is a mild mutation and that other individuals homozygous for this mutation might be unnoticed and misdiagnosed. Low clinical alteration would be consistent with the position of the Met645Arg substitution in the ATP7B protein, between the sixth copper-binding and the first transmembrane domains, a region not essential for the protein function although it can impair copper transport.

P0852. Polymorphisms of RET gene involved in aetiology of Hirschsprung disease

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Hirschsprung disease (Hd) is a congenital disorder, characterized by the absence of intestinal ganglion cells. Various genes are included in aetiology of Hirschsprung disease. Diverse models of inheritance, co-existence of genetic disorders and chromosomal aberrations and involvement of various genes confirm genetic heterogeneity of Hd. It seems that *RET* gene plays the most important role in its aetiology. There are a variety of mutations showed in this gene resulting in a clinical heterogeneity. Recent advances showed that variants of *RET* polymorphisms are over- or under-represented in Hd populations.

The aim of the study was to analyse single nucleotide polymorphisms of *RET* gene in several exons. 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. The study group comprised 120 Polish patients. Molecular analyses were performed in 50 cases. Only 4 investigated patients with Hd have a family history. We found a short segment of aganglionic gut in 64%, ultra-short segment in 16% and long-segment in 20%. The 135A and 1296A and 2712G *RET* variants have been shown to be strongly associated with the Hd phenotype. These data suggest a role for *RET* polymorphisms variant in the aetiology of Hd. In nearest future, the genetic tests could determine the severity of clinical picture of Hd and the risk of Hd patients family.

P0853. Asymptomatic hyperCKemia in a case of Danon disease due to a missense mutation in Lamp-2 gene

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Primary lysosome-associated membrane protein-2 (LAMP-2) deficiency is an X-linked disease, characterized by the clinical triad of cardiomyopathy, vacuolar myopathy and mental retardation, previously known as Danon disease. Mutations of *lamp-2* gene have been reported so far in about 20 patients, one of whom was Italian. We describe herein a 23 year-old man with a Wolf-Parkinson-White syndrome and a non-obstructive hypertrophic cardiomyopathy. He was admitted to our department because, in several occasions, blood tests revealed persistently increased serum CK (max 783 IU/L, n.v. <200). A muscle biopsy revealed a vacuolar myopathy with mild glycogen storage and immunohistochemical studies detected LAMP-2 deficiency. A new nucleotide substitution (T961C) on exon 8 of *lamp-2* gene was identified as responsible of the protein deficiency. This is a new Italian case with persistent hyperCKemia, exercise intolerance and hypertrophic cardiomyopathy but with no muscle weakness or mental impairment. Different mutations have been described, but almost all of them determined a truncated not functional protein. In our case the patient harboured a missense mutation so far never described. We suggest that LAMP-2 deficiency has to be included among the causes of asymptomatic hyperCKemia, especially in patients with arrhythmias or hypertrophic cardiomyopathy.

P0854. The relationship between the SMN protein expression level in fibroblasts and the clinical phenotype of SMA patients

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by the loss of motor neurons of the spinal cord. Several SMA forms (types I-IV) have been distinguished on the basis of disease severity and the age of onset. The disease-causing mutations in the *SMN1* gene are thought to affect the level of functional SMN protein. However, although the SMN protein expression level is drastically reduced in fibroblasts of the most severely affected type I patients, similar reduction in type II-IV patients remains a matter of controversy. We examined the protein level in fibroblasts isolated from 27 patients

with different forms of SMA. The Western blot analysis showed that the SMN protein level in affected individuals was reduced by 97.1% in a type I patient, 46 ± 15.6% in type II patients (n=5), 32.7 ± 21.6% in type IIIa patients (n=11), 20.8 ± 16.8% in type IIIb patients (n=9) and 11.3% in a type IV patient. These differences were statistically significant for types I and II, but not for types III and IV patients. Additionally, there was certain overlap between the normal range of expression level and the levels detected in type II-IV patients. Together, these results suggest that tissue-specific regulation of SMN expression in affected cells (motor neurons) as well as other factors may additionally contribute to the observed diversity in clinical manifestation of SMA. Supported by the State Committee for Scientific Research grant 2 P05E 007 27 and the Polpharma grant 201/II/2003.

P0855. Mutation analysis of the *MECP2* gene in Czech, Slovak and Ukrainian patients with Rett syndrome

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Background: Rett syndrome (RTT), is a severe X-linked neurodevelopmental disorder affecting 1/10,000-15,000 females. It is caused by mainly *de novo* mutations in the *MECP2* gene. Its product methyl-CpG-binding protein 2 (MeCP2) binds specifically to methylated DNA and plays an important role in gene silencing. Mutations in *MECP2* lead to decrease/loss of MeCP2 function. To date, more than 200 mutations have been identified in *MECP2* including missense, nonsense, deletions and insertions. We report mutation analysis of 85 girls with RTT from Czech Republic, Slovakia and Ukraine.

Materials and methods: Genomic DNA was isolated from peripheral blood lymphocytes and used to amplify coding sequence and exon/intron borders of *MECP2* gene. PCR products were examined by automated DNA sequencing and RFLP.

Results: The analysis revealed 24 different mutations in 54 sporadic patients (63.5%). Two of them have not been previously published: L108H and P388S. The frequency of mutation T158M compared to other databases (e.g. RettBASE 9.25%) has been much more higher in our patients (20%) and further investigations will show whether this mutation is prevalent in the Slavonic population.

Conclusions: Our results show molecular heterogeneity in patients with RTT, facilitate the molecular diagnosis of RTT in the Slavonic population, and provide insight into the molecular pathology of RTT. Supported by grant GAUK8/04.

P0856. Molecular diagnosis of dysferlinopathies in a clinical setting and report of novel *DYSF* mutations

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Dysferlinopathies belong to the heterogeneous group of autosomal recessive muscular dystrophies. Mutations in the gene encoding dysferlin (*DYSF*) lead to distinct phenotypes, mainly Limb Girdle Muscular Dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Due to the clinical heterogeneity, initial Dysferlin protein analysis on muscle sample is essential to orientate diagnosis. However, diagnosis should be confirmed by molecular analysis of the *DYSF* gene.

The large size of the *DYSF* gene (> 150 kbp, 55 exons) makes this a challenging task on a routine basis. Methods for mutation screening are particularly useful in this regard.

Here, we report the results of mutational screening in a cohort of LGMD2B and MM patients, identified using SSCP and/or DHPLC analysis, and subsequent sequencing of detected variants, in a routine diagnostic setting.

In 31 unrelated patients, molecular analysis confirmed the diagnosis of primary Dysferlinopathy. 12 of these patients carry an homozygous deleterious mutation, while 19 patients are compound heterozygotes. Furthermore, one mutation was identified in 18 patients, without identification of a second deleterious allele. Complementary analysis is still ongoing for these patients.

Most of the identified mutations have not been reported before, and are predicted to produce a truncated protein (38%) or one amino-acid substitution (52%).

We did not find evidence for genotype-phenotype correlations. All genetic and phenotypic data were compiled in a newly created database, the "Universal Dysferlin Mutations Database" (UDMD). This database compiles all mutations, either novel or previously reported in the literature, as well as all polymorphisms known in the *DYSF* gene.

P0857. Characterization of a balanced X/autosomal translocation in a female patient displaying a Rett-like phenotype

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We report the study of a female patient with an apparently de novo balanced X/autosomal translocation between the short arm of the X chromosome and the long arm of chromosome 20. This patient developed at 15 months severe infantile spasms which have been refractory to therapy. The first 6-8 months of development were normal but subsequently her development slowed and she now displays a profound developmental delay.

Cytogenetic characterization using fluorescent in situ hybridization showed disruption of an unknown transcript in Xp11.

The pattern of expression of this transcript was evaluated by RNA *in situ* hybridization on sections from murine embryos at different developmental stages (E12.5-E14.5-E16.5 and P0). Our preliminary study revealed expression of this transcript in the olfactory bulb, the cerebral cortex, the cerebellum, the hippocampus, and the spinal cord.

Considering that the phenotype observed in the patient, closely resembles the phenotype described in Rett syndrome (RTT), we decided to evaluate this gene as a candidate for RTT. Mutation analysis is ongoing in a cohort of 40 cases who had been diagnosed with classical RTT syndrome or with a variant of RTT. Mutations in the methyl-CpG-binding protein 2 (MECP2), the major gene of RTT, and the cyclin-dependent kinase-like 5 gene (CDKL5), responsible for early seizure variant of RTT have both been excluded in this cohort of patients.

Data will be presented on the complete characterization of this transcript.

P0858. Genetic analysis of the coagulation factor VIII and IX genes in Hungarian patients with haemophilia

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Haemophilia A (HA) and haemophilia B (HB) are common X-linked bleeding disorders resulting from the inherited deficiency of coagulation factors VIII (fVIII) and IX (fIX), respectively. Female relatives of patients with haemophilia may be carriers and many of them request carrier status determination. Previously, large gene inversion detection in HA patients and linkage analysis using intragenic polymorphisms in HB and in inversion-negative HA patients were used for carrier and prenatal diagnosis in our laboratory, but in some cases linkage analysis had limitations. A high number of different mutations can be identified by direct sequencing of the fVIII and fIX genes, which is now the preferred method for genetic counseling. The aim of our study was to identify the disease causing mutations in 15 severe HA-patients with no intron inversions and in 20 Hungarian HB-patients by sequencing the promoter and the exons with flanking regions of the fVIII and fIX genes and to provide precise carrier status determination in the affected

families. To date the sequencing of exons 1, 2, 3, 4, 5, 7 and 13 of fVIII gene was completed. We found three novel point mutations (Lys48Gln, Trp68STOP, His256Arg) and one published missense mutation in fVIII gene. Among 20 HB-patients, the entire coding region was sequenced and one novel (Asp64Val) and 17 published fIX gene mutations were found. This new mutation was detected in two unrelated Hungarian families. Our results further confirm, that HA and HB can be caused by a wide variety of point mutations.

P0859. HMG-Box deletion of the SRY gene in two sisters with 46XY gonadal dysgenesis

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Mutations in the SRY gene, which is involved in human sex determination, result in XY sex reversal and pure gonadal dysgenesis.

We report on a familial case of 46 XY pure gonadal dysgenesis in two sisters, 12 and 13 years old, which was fortuitously detected after the development of dysgerminoma and gonadoblastoma in the younger one, at the age of 11 years old. The tumor originated from the right gonad which was surgically removed. The left gonad and the tubes were not removed at this time. Both sisters present unambiguous female external genitalia, normal size of uterus and streak gonads. Hormonal studies are consistent with hypergonadotropic hypogonadism. The oldest sister remains asymptomatic.

Methods: Genomic DNA was extracted from peripheral blood lymphocytes of both individuals. Molecular analysis of Y chromosome was performed by PCR amplification of the SRY and ZFY loci as well as the AZF region.

Results: A deletion within the SRY gene which includes the HMG-box was revealed in both sisters.

Conclusions: Molecular analysis is consistent with the diagnosis of pure gonadal dysgenesis. The remaining ovary of the youngest sister was removed due to the high risk of developing a new tumor and gonadoblastoma was histopathologically confirmed in the removed gonad. Bilateral gonadectomy was recommended to the oldest asymptomatic sister in order to avoid the development of a gonad tumor in the future.

Since the deletion is detected in both patients the case is characterized as familial and the possibility of paternal gametic mosaicism should be considered.

P0860. Molecular-genetic study of Russian PKU patients

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Phenylketonuria is an autosomal recessive human genetic disease caused by mutations in the phenylalanine hydroxylase (PAH) gene. To date more than 400 mutations have been described. This disease is associated with severe mental retardation.

We studied 296 PKU patients from different Russian regions. For screening of 8 most frequent mutations in exons 3, 5, 7, 11 and 12 of the PAH gene a multiplex system for ACES PCR analysis was developed. This system detected 78.3% mutations in our patients. For other PKU chromosomes PCR-SSCP analysis of all PAH exons and intron/exon junctions with sequence analysis of fragments with altered electrophoretic mobility was performed. We have revealed 28 different mutations, including 4 novel mutations (I421N, G256D, Ala132Val, del865-872nt) that represent 86.8% of all defective chromosomes. Two patients from our group have three mutations in their genotype. One of them has R408W on one chromosome and (IVS10-14c->g+ IVS 11+1g->c) in *cis* on the other; the other patient has P281L on one chromosome and (A132V +del865-872nt) in *cis* on the other. Our approach failed to reveal any PAH mutations on 78 PKU chromosomes (13.2%). This group includes 60 chromosomes in patients with one confirmed mutation. In 9 PKU patients we have not found any mutations in the PAH gene and suppose that they may have a disorder in the QDPR gene. Investigations of this gene are in progress now.

P0861. Cryptic genomic rearrangements in Beckwith-Wiedemann patients.

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Beckwith-Wiedemann syndrome (BWS; MIM 130650) is a developmental disorder, characterized by prenatal and postnatal overgrowth, macroglossia and anterior abdominal wall defects. The etiology involves genetic and epigenetic alterations affecting the 11p15 region, which is organised into two imprinted domains under the control of DMR1, 4Kb distal to 5'H19, and DMR2 in KVLQT1 IVS10. Only 2% of BWS patients were found to carry cytogenetics abnormalities. A cohort of 60 BWS patients was investigated for the most common pathogenetic mechanisms: 11p15 mosaic paternal UPD and defects in methylation pattern of the imprinted H19 and LIT1. Microsatellite segregation analysis, carried out to search 11patUPD, showed in two patients a profile compatible with the presence of cytogenetic rearrangements.

FISH analysis confirmed that the first case, trisomic for D11S318 and showing both paternal methylated alleles at H19, carries de novo dup(11)(p15.5p15.5), likely resulting from unequal recombination at meiosis I in paternal gametogenesis. The second patient carried a der(21)(11;21)(11p15.4;q22.3) originated from missegregation of a cryptic paternal translocation. An additional patient referred to have Wolf syndrome with unusual height was shown to carry a t(4;11)der(p16.3;p15.4) and characterised by FISH analysis. Interestingly, refined breakpoint mapping evidenced that both translocations affecting 11p15.4 are mediated by segmental duplications.

P0862. Connexin 26 mutation 35delG in nonsyndromic sensorineural hearing loss in Latvia: preliminary findings

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Congenital profound hearing impairment affects about one in 1,000 neonates; half of these cases are due to genetic factors. Approximately 80% of all hereditary deafness cases are classified as nonsyndromic and recessive. So far 46 genes that, if mutated, result in nonsyndromic recessive hearing loss have been identified. Mutations in GJB2 gene, which encodes a gap-junction beta-2 protein (connexin 26), are the main cause of nonsyndromic sensorineural autosomal recessive deafness. Connexin 26 is a transmembrane protein that forms intracellular channels known as connexons. These channels regulate cell metabolism, differentiation, and the transmission of the electrical impulses. The 35delG mutation is the most common mutation in the GJB2 gene in many populations.

In Latvia there were registered 1613 children with hearing impairments in May 2004 and every year about 150 more are born. We obtained DNA samples from patients with nonsyndromic sensorineural hearing loss and their relatives. During the period from December 2003 till May 2004 19 patients were screened for 35delG mutation, 13 (68%) are heterozygous for this mutation. One patient (5%) has genotype 35delG/N or 35delG/X, where X is unknown mutation, possibly he is a compound heterozygote. For 5 patients (26%) there was no 35delG mutation found. Eight from 14 relatives of the screened patients found to be heterozygous for the searched mutation. We are planning to continue 35delG screening as well as to analyze heterozygotes and 35delG unaffected patients by direct sequencing to reveal full spectrum of mutation in GJB2 in Latvian patients.

P0863. Characterization of novel mutations in the CYP1B1 gene in Mexican patients with primary congenital glaucoma

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Mutations and polymorphisms have been identified in the CYP1B1 gene, while mutations that affect the conserved core structures

of cytochrome P4501B1 result in primary congenital glaucoma, mutations in other regions hold the potential to define differences in the estrogen metabolism. In the present study, we analyzed the CYP1B1 gene in Mexican patients with primary congenital glaucoma (PCG) and described four novel mutations and a novel allele variant of the CYP1B1 gene. Sample included 12 non-related cases with PCG. Analysis of coding regions of the CYP1B1 was performed through PCR and DNA sequencing analysis from genomic DNA. Molecular analysis of the CYP1B1 gene showed the following molecular defects: 1) a novel single base pair deletion within codon 370 (1454delC); 2) a novel single base pair deletion within codon 277 (1176delT) 3) a novel single base pair deletion within codon 179 (880delG); 4) a duplication (or insertion) of ten base pairs within codon 404 (1556dupATGCCACAC) and a novel polymorphic variant of the cytochrome P4501B1 with six single nucleotide polymorphisms. We observed that deletions and insertions are the principal molecular defects of the CYP1B1 gene in PCG in Mexican population, it has important implications in diagnosis and genetic counseling of this population.

P0864. Mutation analysis in the DCX gene confirms the high prevalence of mosaicism as a cause of subcortical band heterotopia / double cortex syndrome

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Mutations in the X-linked gene doublecortin (DCX) are the cause of X-linked lissencephaly in males and X-linked subcortical band heterotopia (SBH, double cortex syndrome) in females. We have performed mutation analysis in the DCX gene in 19 patients (15 females, 4 males) with double cortex syndrome, by sequencing the entire coding region to look for small intragenic mutations and performing Southern blotting to look for larger deletions or duplications. We have found small intragenic mutations in 6 of the female patients, and in one male patient. 3 of the mutations are new: two missense mutations in exon 4 (N60D and T88R) and a 3 bp deletion in exon 5 (c.648-650delAGA) which leads to the deletion of a lysine residue. The previously described mutation, c.684-685delCT was found to be heterozygous in a 51-year old male patient with SBH. The patient had no family history of lissencephaly and sequence analysis in both his parents and healthy brother and sister did not reveal the DCX mutation. The possibility of an extra X chromosome was eliminated via chromosome and FISH analysis. The inheritance of a single X chromosome from the patient's mother was demonstrated via fragment analysis of the CAG repeat in the HUMARA gene on Xq11-q12. The most likely conclusion is that the patient is mosaic for the c.684-685delCT mutation. Somatic mosaicism has previously been reported in 4 male patients with double cortex syndrome, and although a relatively rare event, should not be overlooked as a possible cause in males with a similar phenotype.

P0865. The 2P16 deletion syndrome: Characterization of the transcription content

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The vast majority of small deletions syndromes, involving several genes, are caused by haploinsufficiency of one or several genes and are transmitted as dominant traits. We have previously identified patients originating from an extended family presenting a unique syndrome, inherited in a recessive mode, consisting of cystinuria, neonatal seizures, hypotonia, severe somatic and developmental delay, facial dysmorphism, and lactic acidemia. Reduced activity of all the respiratory chain enzymatic complexes that are encoded in the mitochondria was found in muscle biopsy specimens of the patients examined. The molecular basis of this disorder is a homozygous deletion of 179,311 bp on chromosome 2p16, which includes the type I cystinuria gene SLC3A1, the protein phosphatase 2C beta gene, an unidentified gene (KIAA 0436) and several ESTs. We now present the transcription content of this region: Each of the three previously reported genes encodes multiple, previously unidentified, splice variants. In addition two transcripts with no open reading frame and

a short, predicted gene, that encodes a putative protein of 67 a.a. not similar to any other protein are encoded in the region. The first exon of an additional gene (*FLJ23451*) is encoded in the deleted region and the gene is not expressed in the patients. The absence of the deleted genes cause the specific clinical phenotype observed in the 2p16 patients.

P0866. Inheritance of novel *LMNA* and *ZMPSTE24* mutations associated to segmental ageing syndromes.

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Several segmental progeroid disorders are due to mutations of at least two genes, *LMNA* and *ZMPSTE24*, respectively encoding Lamins A/C, ubiquitous and multifunctional nuclear proteins, and a metalloprotease involved in post-traductional Prelamin A processing. In the absence of mature Lamin A with accumulation of Prelamin A, or in the presence of truncated Lamin A forms, segmental progeroid syndromes seem to develop. We report here three familial cases of segmental progeroid syndromes carrying mono-, di- or tri-allelic inherited mutations. In one consanguineous family, two sibs were affected with progeria associated to neonatal signs. Only a retrospective study was possible, since no biological material was available from the affected sibs. The parents carried both a heterozygous *ZMPSTE24* c.1053_1055del and the mother carried in addition a heterozygous *LMNA* c.1643G>A (p.R545H). We infer that the two affected sibs carried the homozygous *ZMPSTE24* deletion and the heterozygous *LMNA* substitution. In another consanguineous family, the index case, affected with neonatal progeria, inherited from the father and the mother respectively, heterozygous IVS8+6T>G and Lamin A-specific c.1961_1962insG. Western Blot studies from fibroblast cultures showed a reduction of Lamin A levels. A third patient, affected as his father with a localised form of acrogeria, carried a novel heterozygous *LMNA* substitution, c.1771T>A (p.C591S), specifically affecting as well Lamin A. These results confirm a major involvement of Lamins A/C and functionally related proteins in segmental progeroid disorders, predicting the existence of a much larger series of allelic disorders, and underscore the need to expand molecular and functional explorations on affected patients.

P0867. Detection of a new complex allele associated with HFE 1 hereditary haemochromatosis

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The majority of type 1 haemochromatosis (HH) patients in North of France are homozygous for the missense mutation 845C à A (C282Y) in the HFE gene. About 5% of patients are compound heterozygotes for C282Y and a second common missense mutation 187CàG (H63D). The effect of this second allele is much less severe than the C282Y mutation as it does not prevent the cell surface localization of the HFE protein. Depending on the population studied, from 4 to 35 % of patients presenting with the HH phenotype carry only a unique C282Y or H63D allele or none at all. HH in such patients suggests genetic and/or allelic heterogeneity. Indeed, rare HFE gene mutations have been documented that are mostly only associated with haemochromatosis when found in conjunction with either H63D or C282Y.

In a patient presenting with iron overload (ferritin 1900 mg/L ; transferrin saturation 94%) we first identified a new missense mutation : G43D which was shown to segregate within the family with the allele also carrying the H63D mutation. The association of the G43D mutation in cis-combination with H63D mutation produces a complex allele with both the mutations in the same protein domain. Molecular modelling studies were in favour of a destabilizing effect of the G43D mutation within the a1 domain of the HFE protein. We conclude that the G43D/

H63D complex allele has consequences as severe as that of the C282Y allele and thus that G43D-H63D/C282Y genotype is responsible for the disease

P0868. Molecular analysis of myotonic dystrophies DM1 and DM2 in Czech Republic.

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Myotonic dystrophies are the most common form of adult muscular dystrophy with estimated incidence of 1/8000. It can be caused by a mutation on either chromosome 19q13 (DM1) or 3q21 (DM2/PROMM). The DM1 is caused by an unstable CTG repeat expansion in the 3' untranslated region of the dystrophia myotonica-protein kinase gene (DMPK) and DM2 is caused by a CCTG expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene.

We present a cohort of 426 patients with the myotonic dystrophy. We confirmed the diagnosis DM1 by the molecular genetic analysis in the 121 patients (53 families). DM1 negative patients (142) were already tested for the DM2 mutation. The DM2 diagnosis was excluded in 128 of them and 6 patients (5 families) had an CCTG expansion in ZNF9 gene. We also found a patient with suspected myotonia and with a premutation and another patient with the myotonic dystrophy diagnosis who has both alleles of DMPK gene expanded with the different number of CTG repeats.

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P0869. Our first experience in preimplantation genetic diagnosis

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Prevention of the birth of affected children in couples at risk for transmitting a genetic disorder is currently based on population screening and prenatal diagnosis, followed by termination of affected pregnancies. However, this is the most sensitive problem in the control of genetic disease, which is incompatible with life or not tolerated in populations and ethnic groups.

Preimplantation genetic diagnosis (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 at risk. PGD may be achieved by testing female gametes (polar body analysis), blastomeres from cleavage stage embryos, or blastocysts. PGD can be applied for monogenic disorders or chromosomal abnormalities, using diagnostic protocols based on the polymerase chain reaction (PCR) or fluorescence in situ hybridisation (FISH), respectively.

Our genetic centre at present concentrates on chromosomal abnormalities, while also on genotyping for single-gene disorders. Genetic analyses are performed on biopsied blastomeres.

PGD of chromosomal abnormalities is applied in patients with repeated IVF failures or spontaneous abortions. This application is essentially a screening procedure to detect aneuploidies/polyploidies or translocations most commonly observed postnatally or in spontaneous abortions.

We optimise the first PCR based PGD protocol for genotyping of Cystic Fibrosis Transmembrane Regulator (CFTR) gene with focusing on several inherent difficulties associated with single cell DNA amplifications including potential sample contamination, total PCR failure and allelic drop out. These difficulties should be minimised for any PGD PCR protocol before clinical application.

P0870. A novel exonic mutation triggers mRNA degradation in a GM1 gangliosidosis patient

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GM1 gangliosidosis is a lysosomal storage disease caused by a hereditary deficiency of lysosomal acid beta-galactosidase noted by visceromegaly and neurologic symptoms.

Three major clinical phenotypes are distinguished in GM1 gangliosidosis: infantile, juvenile and adult.

A mutational analysis in a large cohort of GM1 Spanish patients was performed. Here we present the characterization of a novel mutation found in an infantile patient. After completely sequencing the cDNA of the patient, only one mutation (c.1491G>T; p.G481X) was found. To identify the second mutation, the genomic DNA was sequenced. The nucleotide substitution (c.952C>T), in exon 8, was found. The fact that this substitution appeared in the genomic DNA but not in the cDNA suggested that the change could trigger the degradation of the mRNA by nonsense mediated decay (NMD), a mechanism that clears mRNAs containing premature termination codons (PTC).

Skin fibroblasts of the patient were cultured with cycloheximide (CHX), a known inhibitor of protein synthesis and of NMD. The sequencing of the cDNA from the treated fibroblasts revealed the presence of an additional transcript. It contained a deletion of the last 14 bp of exon 8 resulting in the generation of a PTC that elicits NMD on the novel transcript. The c.952C>T mutation in exon 8 is in fact a splicing mutation that affects the normal splicing by creating a novel 5' donor splice site with a higher splice site score than the usual one. A minigene assay was also used to confirm the results.

P0871. A new mutation causing multiple sulfatase deficiency

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Multiple sulfatase deficiency (MSD) is a rare autosomal recessively inherited lysosomal storage disorder characterized by the accumulation of sulphated lipids and carbohydrates. The disorder combines features of metachromatic leukodystrophy and of mucopolysaccharidosis. Increased amounts of acid mucopolysaccharides are found in several tissues. The defect in this disorder involves a posttranslational process, common to 7 or more sulfatases (Rommerskirch et al., 1992) and decrease the capacity of a process that renders sulfatases enzymatically active or prevents their premature inactivation. C-alpha-formylglycine (FGly), the catalytic residue in the active site of eukaryotic sulfatases, is posttranslationally generated from a cysteine in the endoplasmic reticulum (ER). The *SUMF1* gene encodes FGly-generating enzyme (FGE) and is mutated in MSD. Dierks et al. (2003) and Cosma et al. (2003) identified several mutations in the *SUMF1* gene in patients with MSD.

Here we present the mutation analysis of 3 MSD patients, two from Spain and one from Argentina. The first patient (MSD1) is homozygous for a 463T>C substitution (S155P) and the second patient (MSD2) is homozygous for a 1033C>T substitution (A345C). These two mutations were previously described by Cosma et al. (2003). The third patient (MSD3) is homozygous for a novel mutation, IVS7+5 G>T, which affects the splice donor site of intron 7. Since no sample from this patient was available, we used a minigene approach to analyse the change and confirmed that it produced an aberrant splicing.

P0872. Characterization of CREBBP deletions in Rubinstein-Taybi patients using array-CGH

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The Rubinstein-Taybi syndrome (RTS) is a rare autosomal dominant disease that is associated in 10-15% of cases with 16p13.3 microdeletions, all involving the CREB-Binding Protein gene

(CREBBP). Various techniques used so far to identify these deletions (FISH, microsatellite analysis or quantitative PCR) all suffer from several drawbacks. Due to the high heterogeneity of deletions in terms of both size and location inside the gene, array-CGH seems to be a very appropriate technique to analyse CREBBP deletions. We therefore constructed a microarray carrying various types of genomic resources derived from the 16p13.3 region, including 7 BACs and 35 cosmids (spanning 2 Mb). In order to visualize deletions as small as 10-20 kb, a set of 28 low molecular weight probes (800-1500 bp) was also designed, that are spread along the CREBBP gene at an average density of one clone every 6 kb.

In the present study we show that this microarray was able to visualize all previously known deletions and to refine the position of the deletion breakpoints in some cases. We also demonstrated that targets as small as 1000 bp can be used efficiently to detect heterozygous deletions. This constitutes the first example of the use of this kind of small targets for a region- or gene-specific microarray as a tool for deletion identification in a genetic disease, and paves the way for a wider use of the technique as a diagnostic tool, especially since a high level of resolution can be attained.

P0873. Variability of disorders caused by mutations in *LMNA* gene

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LMNA gene encoding two proteins, lamins A and C, is responsible for about fifteen various disorders named laminopathies. We have studied 87 unrelated families with different laminopathies including Emery-Dreifuss muscular dystrophy (EDMD), myodystrophy with cardiomyopathy, dilated cardiomyopathy type 1A (DCM), autosomal recessive congenital polyneuropathy, limb girdle muscular dystrophy (LGMD), progeria, lipodystrophy. A search of *LMNA* gene mutations and polymorphisms was performed by SSCP method with follow sequencing. We have found eight different missense mutations (Asp47His, Gly232Arg, Arg249Gln, Arg541His, Ala350Pro, Asn459Tyr, Gly635Asp, delLys261+ins15bp) in nine families. Fore families presented autosomal dominant EDMD including one with atypical severe course. In one case LGMD type 1B was diagnosed. Three families had DCM, including atypical early variant in one. Autosomal recessive congenital polyneuropathy in two sibs being different from HMSN type 2B1 may present a novel, previously undescribed phenotype. Seven found mutations were novel. Arg249Gln mutation previously described in three unrelated patients with typical EDMD was detected in two unrelated families with different clinical phenotypes, namely LGMD type 1B and mild EDMD. This fact points to existence of relatively frequent *LMNA* mutations and also to a role of some modifying factors in phenotype formation. Also we have found seven different polymorphisms in the coding region of *LMNA* gene. Two undescribed polymorphisms, G824A and G1973A, don't lead to aminoacid substitution but may cause the alternative splice site formation according to computer programme predicted exon-intronic gene structure. The population frequency of these polymorphisms is studied now.

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P0874. Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency: Biochemical and mutation analysis in Latvia

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Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an autosomal recessive disorder of mitochondrial fatty acid oxidation (MIM# 143450). The LCHAD enzyme is part of the mitochondrial trifunctional protein (MTP). A common point mutation 1528 G>C (E510Q) in the gene coding for the α -subunit of the MTP harbouring LCHAD activity is found in 87%-90% of the alleles of patients.

We have two confirmed cases of LCHADD in our centre. Both were confirmed with the kind help of colleagues abroad - Prof. Sass (Metabolic Unit, University Children's Hospital Freiburg), Prof. Zschocke (Institute of Human Genetics, Heidelberg), Dr. Huijmans (Erasmus University,

Rotterdam).

Case 1 was diagnosed using biochemical methods in the Netherlands, mutation analysis was performed in the Latvian State Medical Genetics Centre (LSMGC) at the end of 2004. Case 2 was screened biochemically in the LSMGC, the diagnosis of LCHADD was confirmed with biochemical analysis and mutation analysis in Germany. Mutation analysis for parents of both LCHADD patients was performed in our centre.

During biochemical screening for organic acid analysis by gas chromatography (GC) for LCHADD patient C6-C10 dicarboxylic aciduria was found (adipic acid↑, suberic acid↑, sebamic acid↑) in our laboratory. The results of organic acid screening in urine had led us to a diagnosis of defects in fatty acid beta-oxidation.

The molecular studies revealed that both of our patients are homozygous for the 1528 G>C mutation. PCR-RFLP was used for mutation analysis. We suggest that mutation analysis for common 1528 G>C mutation should be performed for patients with positive biochemical screening.

P0875. Abnormal microvilli function in autosomal dominant hearing loss due to mutations in the espin (ESPN) gene

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Espins represent a family of multifunctional actin cytoskeletal regulatory proteins with the potential to differentially influence the organization, dimensions, dynamics, of the actin filament-rich, microvillus-type specializations that mediate sensory transduction in various mechanosensory and chemosensory cells; they are associated with the parallel actin bundles of hair cell stereocilia. The espin COOH-terminal peptide, which contains the 116-amino acid actin-bundling module, is necessary and sufficient for the microvillar lengthening activity. This ability to increase microvillar length in transfected epithelial cells has led to the hypothesis that espins play an essential role in determining the length of hair cell stereocilia. In support of this hypothesis, espin protein level is correlated with stereocilia; a recessive mutation in the espin gene has been detected in the jerker mouse and causes deafness and vestibular dysfunction and hair cell degeneration. More recently ESPN mutations have been described in two families affected by autosomal recessive hearing loss and vestibular areflexia. Here, we report the identification of 4 additional ESPN mutations in patients affected by autosomal dominant hearing loss. To determine whether ESPN mutated alleles affected the biological activity of the corresponding espin proteins *in vivo*, we investigated their ability to target and elongate the parallel actin bundles of brush border microvilli in transfected LLC-PK1-CL4 epithelial cells. For three mutated alleles clear abnormalities in microvillar length or distribution were obtained. These findings further strengthen the causative role of the espin gene in nonsyndromic hearing loss and add new insights in our knowledge on ESPN activity in the hearing system.

P0876. Rare and frequent sequence variation in PKAN and the correlation with neurodegeneration

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Panthotenate kinase-associated neurodegeneration is a very rare autosomal recessive enzyme deficiency. The disorder is characterized by a progressive loss of neurons mainly affecting cells in the basal ganglia and the eye. Both missense and nonsense mutations have been described in the hPKAN2 gene, one of the four highly homologous kinases which are predicted to catalyse the first step on CoA-biosynthesis. Recently, we have shown that an alternative PKAN2 transcript is coding for a mitochondrial isoform of the enzyme. In an attempt to improve the mutation detection rate and to predict the activity of the enzyme, we now determined both rare and frequent

variants in 80 patients with a clinic diagnosis PKAN and in a sample of 800 controls.

Apart from missense and nonsense mutations, we also found a small proportion (5%) of deletions. There was no one to one correlation between PKAN2 mutations and the radiological sign of an "eye of tiger" as repeatedly reported in the literature. No mutation was detected in 35% of cases which strongly argues for non-allelic heterogeneity of the disorder. Mainly the age of onset, but to a lesser extent the course of the disease is negatively correlated with the rest activity of the mitochondrial PKAN2 isoform. The PANK2 activity was predicted from a completion assay in *E. coli*.

A coding polymorphism in exon 1 shows a markedly reduced activity and is found to be heterozygote (50/800) and homozygote (7/800) in the general population with potential consequences for therapeutic options provided by substrate supplementation.

P0877. A CYP21B/CYP21P chimeric molecule as a possible cause of nonclassical form of Congenital Adrenal Hyperplasia

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Congenital adrenal hyperplasia (CAH) is a monogenic autosomal recessive disorder; deficiency of 21-hydroxylase (21OH) accounts for 90-95% of cases. The structural gene (CYP21B) for 21OH is in the HLA class III region on the short arm of chromosome 6 (6p21.3), as also is the pseudogene (CYP21P). This region of the genome is subject to unequal crossover events and gene conversions because of the high homology and tandem-repeat organization of both genes. The region between intron 2 and the 3'end of exon 3 in CYP21 is considered as a hotspot for recombination and microconversion.

We previously reported that a novel CYP21B/21P hybrid gene was found at the 6p21.3 locus. It is characterized by a junction site after exon 8 and differs from the normal CYP21 gene in its 3'-region, which corresponds to the CYP21P pseudogene.

Using PCR we analyzed the frequency of CYP21B/21P and CYP21P/21B hybrid genes in 33 unrelated Russian CAH patients with nonclassical forms of CAH-21OH deficiency, in 19 patients with idiopathic androgenization and in 29 controls.

The frequencies of both genes in the patients with nonclassical CAH (32% and 12% respectively) and with idiopathic androgenization (16% and 3% respectively) were significantly higher than in the controls (3.5% and 0% respectively ($p<0.05$)).

A chimeric CYP21B/21P gene might be suggested as a feasible cause of the nonclassical form of CAH. The patients with idiopathic androgenization, possessing this gene might be considered as having a nonclassical form of CAH.

P0878. Molecular characterization of a t(2;6) balanced translocation associated with complex phenotype and leading to the truncation of the TCBA1 gene

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Molecular characterization of chromosome rearrangements associated with abnormal phenotypes has often provided crucial clues for the positional identification of disease-associated genes. In several cases, these rearrangements may be associated with a variety of different phenotypes, thus establishing a genotype-phenotype correlation might be quite complex. Nevertheless, the molecular characterization of the rearrangement remains of help either in providing tools for diagnosis, or for the prognosis or finally in establishing a gene to function relationship. We have recently investigated a de novo balanced translocation t(2;6)(q24.3;q22.31) found in a patient with a complex phenotype. The major clinical finding was an epileptic encephalopathy associated with cerebral atrophy involving the periventricular white matter, spastic tetraparesis and severe psychomotor retardation. The molecular characterization of this translocation showed that the truncation of the TCBA1 gene on 6q had occurred. We found that this gene is transcribed in different splice variants and is highly specific for the central nervous system. TCBA1 does not show any similarity

with other known genes and its function is unknown. However, it appears to be well conserved among species and we were able to infer the sequence of a putative mouse homologue of TCBA1. This allowed us to perform a more detailed expression study in mouse thus confirming its specificity for the nervous system. This is of particular interest because it suggests that TCBA1 could be correlated with the neurological phenotype of our patient and possibly mutated in genetic diseases with a similar neurological phenotype.

P0879. MBL2 genotypes and the risk of premature birth

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Allelic variants in the mannose-binding lectin 2 (MBL2) gene are associated with increased risk of infections in newborns or immune deficient patients. Recently, women if carriers of the common codon 54 variant were found to have a higher risk of preterm delivery. Here, we evaluated if the genotype of the child might contribute to the risk of premature birth. By applying an on-chip PCR genotyping method, suitable for the simultaneous detection of common variants (codons 52, 54, and 57; promoter -551 and -220), we tested 204 genomic DNA samples, isolated from blood spots on Guthrie cards. The panel of polymorphisms allowed the exact assignment of genotypes to either MBL wild-type (A), MBL-deficient (0), or low MBL-producing haplotypes (LXA). The MBL2 genotype of children born before the 35th week of gestation (n=102) was compared to a that of a term birth control group (n=102). The codon 52 variant was significantly overrepresented in the group of preterm birth (prevalence of variant allele = 11% vs. 5%), whereas the codon 54 polymorphism was not (12% in both groups). The rare codon 57 polymorphism was also more common in the case group (2% vs. 1%). Furthermore, a biochemical phenotype of potentially low MBL levels assigned to the 0/0, XA/0, and XA/XA haplotypes was also significantly more frequent in the group of premature birth compared to the controls (22.5% vs. 9.8%). Our results suggest that the MBL2 genotype of the unborn child might contribute to the risk of premature delivery.

P0880. Predominance of splicing mutations in the central Fanconi anemia gene FANCD2

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Fanconi anemia (FA) is a recessive genetic instability disorder characterized by congenital abnormalities, bone-marrow failure, sensitivity to crosslinking agents, and cancer susceptibility. To date, nine FANC genes have been identified which interact in the recognition and repair of DNA-damage. We screened FA patients excluded from complementation groups FA-A, -C, D1, E, F, G or L for mutations in FANCD2, the central effector protein of the FA/BRCA pathway. Even though mutation analysis of FANCD2 is complicated by the presence of pseudogene regions, we identified biallelic FANCD2 mutations in 25 of 26 patients. The predominant types of mutations result in aberrant splicing causing exon skipping, exonisation of intronic sequence, activation of cryptic and creation of new 3' splice sites. Exon 22 skipping caused by two different base substitutions in the polypyrimidine tract of the splice acceptor was prevalent in patients of German and Turkish origin. Three other patients showed skipping of exon 5 caused by insertion of an Alu repeat into the preceding intron sequence. Except for 36 missing nucleotides (1 to 36), the inserted Alu element was identical with Yb9. The mutation in the newly created donor sequence causes a change of donor strength from low to high complementarity, and thus in the recognition of a new exon. Among the 9 known FA genes, only FANCD2 shows this high prevalence of splicing mutations.

P0881. Preliminary data suggest that mutations in the CgRP pathway are not involved in human sporadic cryptorchidism

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In testicular descent to the scrotum, a multistep process, many anatomical and hormonal factors play a role. Cryptorchidism occurs in about 1-2% of males and may cause secondary degeneration of the testes. Animal models have shown that abnormalities, in the calcitonin gene-related peptide (CgRP) activity, could be relevant in the pathogenesis of cryptorchidism. We performed a mutation screening by PCR exon amplification, single-strand conformation polymorphism (SSCP) and sequencing in four candidate genes, CgRPs (α CgRP, β CgRP), their receptor (CgRPR) and the receptor component protein (CGRP-RCP), in 90 selected cases of idiopathic unilateral or bilateral cryptorchidism. Mutation screening of the coding regions and intron-exon boundaries revealed some polymorphic variants but no pathogenic sequence changes. These preliminary data suggest that these genes are not major factors for cryptorchidism in humans.

P0882. Mutations in the glucocerebrosidase gene in Ashkenazi patients with Parkinson's disease: genotype-phenotype correlation analysis

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Background: Mutations in the glucocerebrosidase (GBA) gene have been recently identified as contributory to parkinson's disease (PD), in Ashkenazi Jews. The objective of the present study was to evaluate whether the clinical characteristics of PD patients, carriers of a GBA mutation differ from those of non-carriers.

Methods: A clinic-based case series of 137 Ashkenazi patients with idiopathic Parkinson disease was screened for the six most common GBA mutations (N370S, L444P, 84GG, IVS+1, V394L, R496H) found in Ashkenazi Jews. Family history, demographic and clinical data were obtained using structured questionnaires.

Results: Thirty-seven (27%, 95% confidence interval 19.6-34.4%) of the Ashkenazi PD patients carried a mutated GBA allele. Altogether, 26 N370S heterozygotes, 5 N370S homozygotes, two R496H heterozygote and four 84GG heterozygotes were identified. All PD patients had an initial favorable response to dopaminergic agonists or L-Dopa. GD carriers did not differ from non-carriers with regard to age of disease onset (mean age of disease onset 60.29+13.65 years and 63.15+11.4 years, respectively), gender, family history of PD in a first-degree relative, initial motor manifestations or initial response to levodopa or dopaminergic agonists. Patients with a family history of PD, either GBA carriers or non-carriers, had a significant earlier age of onset (57.08+ 10.57 vs. 62.56+11.22 years, p=0.01).

Conclusions: Sporadic PD patients who carry a GBA mutation manifested typical PD and did not differ clinically from PD patients who did not carry a GBA mutation.

P0883. Usefulness of quantitative techniques using PCR to assess small genomic rearrangements : example of the *Golli-MBP* locus study in hypomyelinating leukodystrophies (HL)

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Leukodystrophies represent a heterogeneous group of rare inherited disorders that involve primarily the white matter of the central nervous system (CNS). The proteolipoprotein (PLP) gene is implicated in X-linked HL. However, only 50% of HL are associated to PLP mutation and as both males and females present HL other gene(s) may be implicated in this disease.

The myelin basic protein (MBP) gene, localized on chromosome 18q, is included in a gene structure called *Golli-MBP* complex and encodes for the second most important class of CNS myelin proteins. As *Mbp* rearrangements have been found in spontaneous dysmyelinating

murin mutants and as patients with a 18q deletion syndrome displayed abnormal myelination pattern on MRI, we looked for *Golli-MBP* rearrangements in 195 HL without PLP mutation.

As preliminary results obtained by FISH in a pre-screening step led us to think that duplication in a mosaic form may exist, we adapted the Multiplex Amplifiable Probe Hybridization (MAPH) and the Quantitative Multiplex PCR of Short Fluorescent fragments (QMPSF) techniques to quantify *Golli-MBP* copy number in our large cohort. The 3 "suspected" duplications could not be confirmed by both PCR-based techniques (eventhough their sensitivity to detect a mosaicism), neither by FISH using a different labeling protocol. In the 185 remaining patients no duplication nor deletion was found. This work highlights the difficulty to use FISH for rearrangement detection (particularly duplications) and allowed us to compare advantages and disadvantages between QMPSF and MAPH. Finally, rearrangements at the *Golli-MBP* locus seem not involved in the etiology of HL.

P0884. *Shox2* acts upstream of *Bmp4* in regulation of sinus venosus and venous valve development

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The striking expression pattern of the homeodomain transcription factor *SHOX2* in the heart of human and mouse embryos suggests its involvement in the early stages of heart development. We have created a null allele of the *Shox2* gene by targeted mutation and here report on fatal heart malformations in *Shox2* homozygous mutant embryos. *Shox2*^{-/-} mouse embryos exhibit retarded development of the sinus venosus region, lack the sinoatrial valves, and develop atrial dilation and thoracic oedema. They die from heart failure between embryonic day 11 and 13. *In situ* hybridization revealed a dramatic down regulation of *Bmp4* expression in the myocardial wall of the sinus venosus and in the proepicardial organ. These observations demonstrate an essential function of *Shox2* upstream of *Bmp4* in the development of the inflow tract region and for the first time demonstrate an indispensable role of the sinus venosus and the venous valves in development of the inflow tract region.

P0885. Screening for mutations in the whole coding regions of *ACVRL1* and *ENG* genes in a group of 88 Italian patients affected with Hereditary Haemorrhagic Telangiectasia.

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Hereditary hemorrhagic telangiectasia (HHT) (OMIM#187300) is an autosomal dominant vascular dysplasia leading to telangiectases of skin, mucosa (frequent complications are epistaxis and gastrointestinal bleeding), and visceral arteriovenous malformations (principally in lung, liver, and brain). Two genes have been so far identified to cause HHT if mutated: *ENG* (OMIM#131195, chromosome 9q34.1), a type III TGF-beta receptor; *ACVRL1* (OMIM#601284, chromosome 12q11-14) a TGF-beta receptor Type I. Wallace and Shovlin in 2000 excluded both genes in a HHT family and demonstrated the existence of a third HHT locus. More recently, Gallione et al. (2004) described a combined syndrome of juvenile polyposis and HHT associated with mutations in SMAD4.

The collaboration between three Italian HHT centers let us collect more than 530 DNA samples from HHT Patients and their relatives. We have more than 150 different families from all Italian regions.

We have screened for mutation by SSCP and/or dHPLC the entire coding region of *ACVRL1* and *ENG* in 88 index cases, each one from different families, who were definitely affected with HHT, according to the Curaçao diagnostic criteria.

We found mutations in 51 subjects (57.96%); 32 of them (62.75%) were mutated in *ACVRL1* gene, 19 (37.25%) in *ENG*. We found 26 different mutations in *ACVRL1* and 17 in *ENG*.

7/32 subject are carriers of mutations in *ACVRL1* never reported by other groups. The same observation can be made for 15/19 *ENG* mutated patients.

We compared the distribution of mutations along the two genes with similar European reports.

P0886. Non Muscle Myosin Heavy Chain II A and II B interact and co-localize in living cells: relevance for MYH9-related diseases

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Myosins of class II constitute part of a superfamily of several classes of proteins expressed in almost all eukaryotic cell types. For class II nonmuscle myosin heavy chains (NMMHCs), three genes, MYH9, MYH10 and MYH14, encoding for NMMHC-A, -B, and -C isoforms, are respectively located on chromosomes 22q12.3, 17p13.3, 19q13.3. Functional studies and knock-out mice on the A and B isoforms, reported differences in their biological properties, suggesting that they might play different biological roles.

Heterozygous mutations of MYH9 cause a complex disorder named MYH9-related disease, characterized by a combination of different symptoms: platelet macrocytosis, thrombocytopenia and leukocyte inclusions, variously associated with sensorineural hearing loss, cataracts and glomerulonephritis. No genotype-phenotype correlation is reported.

In accordance with the clinical picture, NMMHC-A is expressed in platelets, leukocytes, kidney, and cochlea.

A still unresolved problem in MYH9-related disease, is its remarkable symptomatic variability. One hypothesis is that it derives from the joint effect of MYH9 mutations and additional factors, such as polymorphic variants of proteins interacting with NMMHC-A.

These results describe the interaction between NMMHC-A and NMMHC-B, discovered by a yeast two hybrid screening and confirmed by co-immunoprecipitation experiments.

Analysis by confocal microscopy in different cell types showed complete matching of NMMHC-A and NMMHC-B signals in cultured renal podocytes and native bone marrow erythroblasts, whereas partial matching was observed in skin fibroblasts and cultured tubular epithelial cells.

This study provides direct evidences that NMMHC-A and -B interact in vivo by their C-terminal tail region suggesting functional relevance both in physiological and pathological conditions.

P0887. New mutations and a grey zone of CAG repeats in Huntington disease.

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Huntington disease (HD) is one of the group of the neurodegenerative disorders caused by CAG repeats expansion. The main clinical features of this disease are involuntary movements, speech and gait disturbances as well as depression, cognitive decline and dementia. The dynamic mutations may lead to increasing of the CAG repeats number from one generation to another, simultaneously worsening the progression of the disease and decreasing the age of onset. The frequency of HD is estimated for caucasian population for 1: 10.000. The occurrence of new mutation probably depends on the upper non-pathological range of the CAG repeats because it is known that large normal alleles are prone to expansions. Moreover, the range of 36-39 CAG repeats seems to involve incomplete penetrance of the mutated gene.

The aim of our study was to find families with new mutations in *IT15* gene. First, we analysed the polymorphic CAG tract within *IT15* gene and established the normal range for Polish control group (200 chromosomes): 9 to 32 CAG. Then we examined 584 patients of 520 pedigrees with confirmed HD. Here we present two families with confirmed presence of the new mutation and 12 families in whom the grey zone of 36-39 CAG repeats was documented.

P0888. Identification of two mutations in CLC-1 gene in patients with hereditary myotonia

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Non-dystrophic myotonia congenita is an inherited disorder of the skeletal muscle characterized by a delayed muscle relaxation after contraction. Myotonia congenita may be inherited in an autosomal dominant (Thomsen disease) or recessive (Becker disease) manner. The recessive form of myotonia is more frequent and more severe than the dominant one. The both forms of myotonia congenita are caused by mutations in the gene of a skeletal muscle chloride channel (CLC-1) which is located on chromosome 7q35. CLC-1 is important for the normal repolarization of muscular cells membrane. The loss of its function result to the plasma membrane is hyperexcitable and leads to the typical 'myotonic runs' which are seen in electromyograms of patients with myotonia. The study group consisted of 37 families which were mainly from Voronegsky region. In this account 30 probands had clinical diagnosis of Tomsen disease and 7 ones were with Becker disease. We have investigated coding sequencing with exon-intron junction fragments of the CLC-1 gene by PCR-SSCP analyses with follow sequencing of the fragments with alteration of the electroforetic motility. To now we have reviewed heterozygous Thr268Met mutation from the proband with Tomsen myotonia which is published in CLC-1 data based. In armenian family with Becker myotonia we have detected novel mutation Pro342Ser in homozygous. Probably this mutation has armenian origin. The investigation is in process now.

P0889. The importance of non - invasive genetic analysis in the initial diagnostics of Alport syndrome

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Alport syndrome is a hereditary disease, characterized by progressive hematuric nephritis with structural changes of glomerular basement membrane, frequently associated with sensorineural hearing loss. Major changes in glomerular basement membrane are associated with mutations in *Col4a5* which encodes for a5 collagen chain. The disease was also associated with *Col4a3* and *Col4a4*. Alport syndrome has therefore been subdivided into different types: X-linked *Col4a5* (~85% cases) and autosomal recessive *Col4a3* or *Col4a4* disease (~15%). X-linked form is always symptomatic and more severe in affected males (homozygotes). In autosomal recessive form only homozygotes (males, females) develop a progressive disease.

DNA was extracted from peripheral blood of five children aged 10 months to four years. *Col4a3*, *Col4a4* and *Col4a5* were amplified in PCR and analyzed using single stranded conformation analysis and sequenced on ABI PRISM 310 Genetic Analyzer. Clinical data were obtained from clinician who performed precise urine, hearing and ocular examination.

Three novel mutations, G198E, G3189D, and G669R, were detected in *Col4a5*. We assume their pathogenic effect due to glycine key structural role in collagen protein.

In our study we have demonstrated that non-invasive genetic method is a powerful tool in early and precise diagnosis of the disease, specially in young patients. Diagnosis of Alport syndrome in children is not apparent, because the clinical presentation may not always meet proposed strict diagnostic criteria. Screening of three important disease genes (*Col4a3*, *Col4a4*, *Col4a5*) in association with clinical data provide sufficient information for correct diagnosis, therefore generally accepted invasive approaches can be avoided.

P0890. Functional characterization of polymorphic variants in the Osteopontin promoter and their association with diabetic nephropathy

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Osteopontin (OPN) displays several functions in different physiological and pathological processes: bone remodeling, cell-mediated immunity, tissue remodeling during inflammatory processes, tumor

cell metastasis. Overexpression of OPN is the main finding in affected tissues.

Our previous work to understand molecular mechanisms underlying regulation of transcription of human OPN gene lead us to identify and evaluate common variants with functional effect on transcription in the promoter region. We are now investigating the effect of TGF β , an important mediator of inflammatory and immune processes on the OPN promoter and its different haplotype combinations. We describe here an inhibitory effect of TGF β on the OPN promoter: co-transfection experiments showed that TGF β downregulation was mediated by its intracellular mediator SMAD3; no differential response on constructs differing for the variant at -66 in the SP1 site was observed, while this needs to be evaluated for the other sites.

Since both OPN and TGF β are involved in diabetic nephropathy, we carried out an association study in a group of type 2 diabetic patients (112), with and without nephropathy, in which promoter variants, including one at -1747 in a putative SMAD3 binding site, were evaluated for allele frequency in the two diabetic subgroups. We found a significantly different allele frequency of the -1747 variant in diabetic patients with nephropathy compared to non nephropathic ($P=0.015$). These results suggest that OPN promoter polymorphisms affect the gene transcription rate and one of them, located in a target site for the TGF β signaling pathway, is associated with susceptibility to diabetic nephropathy.

P0891. The correlation between insertion in the promoter region of the UGT1 gene and the intolerance of some xenobiotics.

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The *UGT1* gene, which is located on chromosome 2q37, encodes uridine diphosphate glycosyl transferase 1. This enzyme catalyzes the transformation indirect to direct bilirubin in hepatocytes by conjugation with glycuronic acid. Mutations in the *UGT1* gene result in Gilbert syndrome which is characterized by low activity of the enzyme. In patients with Gilbert syndrome use of some drugs leads to an increase of the indirect bilirubin level. The most frequent genetic defect in Gilbert syndrome is a dinucleotide insertion in a TA repeat A(TA)_nTAA in the promoter region of the *UGT1* gene, in homozygous or heterozygous state. In our work we have investigated the correlation between intolerance to medicine for the treatment of Parkinson disease and the presence of the defect in the promoter region of the *UGT1* gene. We have studied 17 patients with extrapyramidal abnormalities who had raised levels of the indirect bilirubin fraction and suffered from iatrogenic hepatitis. We found out that 14 of the 17 patients had the A(TA)_nTAA insertion. Eight patients were homozygous and 6 were heterozygous. The presence of 7 TA repeats in the promoter region of the *UGT1* gene leads to a decrease of the enzyme's activity and this result in disturbance of metabolism of xenobiotics with drug intolerance

P0892. Prevalence of SLC22A4 and RUNX susceptibility SNPs in rheumatoid arthritis patients

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Rheumatoid arthritis is a polygenic inflammatory disease of the joints. A recent study (Tokuhiro et al, Nature Genetics 2003;35:341) suggested involvement of two SNPs as susceptibility factors for the disease: one of them is an intronic polymorphism, called SLC22F (C6,607T change in the first intron of the gene) in the gene coding for the OCTN1 organic cation transporter, the low activity carnitine transporter. This nucleotide position is located within a recognition site for the transcription factor RUNX. The second polymorphism is in the *RUNX* gene (G24658C transversion in the 6th intron). With specific PCR/RFLP methods we investigated the distribution of these two SNPs in Hungarian patients with rheumatoid arthritis (n=209) and in healthy controls (n=217); in addition we determined the plasma carnitine ester profiles by ESI tandem mass spectrometry in the same groups. The allele frequencies of the SNPs did not differ from each other comparing the rheumatoid

arthritis vs. the control groups. The allele frequencies of the Japanese population, as referenced in the original article, significantly differed from that found in the Hungarian subjects. The carnitine ester profiles did not exhibit genotype dependent changes, either in the patients with arthritis or in the control persons. These result strongly suggest that the above SNPs do not mean real susceptibility factors for the rheumatoid arthritis in the Hungarian population.

P0893. Alteration of DNA binding, dimerisation and nuclear translocation of SHOX homeodomain mutations identified in idiopathic short stature and Leri-Weill dyschondrosteosis

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Haploinsufficiency of the short stature homeobox gene SHOX has been found in patients with idiopathic short stature and Leri-Weill dyschondrosteosis. In addition to complete gene deletions and nonsense mutations, several missense mutations have been identified in both patient groups leading to amino acid substitutions in the SHOX protein. The majority of missense mutations were found to accumulate in the region encoding the highly conserved homeodomain of the paired-like type. In this report, we investigated nine different amino acid exchanges in the homeodomain of SHOX patients with idiopathic short stature and Leri-Weill dyschondrosteosis. We were able to show that these mutations cause an alteration of the biological function of SHOX by loss of DNA binding, reduced dimerisation ability and/or impaired nuclear translocation. Additionally, one of the mutations (p.R153L) is defective in transcriptional activation even though it is still able to bind to DNA, dimerise and translocate to the nucleus. Thus, we demonstrate that single missense mutations in the homeodomain fundamentally impair SHOX key functions, thereby leading to the phenotype observed in patients with Leri-Weill dyschondrosteosis and idiopathic short stature.

P0894. Acid CID Alpha Glucosidase Gene: Identification and functional characterization of 12 novel alleles causing the juvenile-adult form of glycogen storage disease type II

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Glycogen Storage Disease type II (GSDII) is an autosomal recessive inherited disorder in which deficiency of acid alpha glucosidase (GAA) results in impaired glycogen degradation that accumulates within lysosomes.

Patients have been traditionally classified, according to disease onset, as infantile, juvenile and adult form. Clinical as well as biochemical heterogeneity have been described in GSDII: the infantile form is the most severe subtype characterized by absent or nearly absent enzymatic activity, progressive muscle weakness and severe cardiac involvement. Juvenile GSDII is associated with reduced enzymatic activity and glycogen storage is generally limited to skeletal muscle. The adult form has clinical signs similar to the juvenile form but residual enzyme activity is higher and miopathy has a slower progression, generally without cardiac involvement.

The GAA gene localizes to human chromosome 17q23; the enzyme is synthesized as an inactive precursor of 110 kDa which is transported to the lysosomal compartment where it is processed into the fully active forms of 76 and 70 kDa. More than 120 mutations in the GAA gene have been described up to date (<http://www.eur.nl/FGG/CH1/pompe/>).

We analyzed the GAA gene in 27 unrelated patients affected of juvenile-adult GSDII. Twelve novel mutant alleles were identified due to 13 novel mutations, two being *in-cis*, on a complex allele. As expected, the IVS1 (-13 T/G) was the most frequent mutation among this group.

Functional characterization of the missense mutations was performed

using a human GAA deficient cell line. All mutant proteins remained as the 110kD precursor expressing no enzyme activity.

P0895. Clinical features of infertile men with androgen receptor mutations and identification of seven novel mutations

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Mutations in the androgen receptor (AR) gene cause a variety of defects related to androgen insensitivity with the less severe phenotype represented by male infertility. However, few studies have analysed the prevalence of AR gene mutations in male infertility and a genotype-phenotype relation is still unclear. We screened for AR gene mutation by sequencing 1517 oligozoospermic men. Of them, 492 were azoospermic, 849 severely oligozoospermic (sperm count < 5 mil/mL), and 176 moderately oligozoospermic (5-10 mil/mL). Clinical evaluation of patients with AR gene mutations included determination of FSH, LH, T and oestradiol, and the androgen sensitivity index (ASI) was calculated. These data were compared to 425 of the 1517 subjects without AR gene mutations and with 310 normozoospermic controls. We found 20 mutations leading to aminoacid changes in 26 of 1517 patients (1.7%), with a similar prevalence in azoospermia, severe oligozoospermia, and moderate oligozoospermia. Of the 20 mutations, 7 represent novel mutations. With respect to idiopathic patients, men with AR gene mutations have lower sperm count (2.2 ± 2.8 vs 3.9 ± 2.6 mil/mL, $P < 0.01$), lower ejaculate volume (2.3 ± 1.2 vs 3.2 ± 1.5 mL, $P < 0.01$), higher T levels (23.8 ± 7.8 vs 15.4 ± 4.8 nmol/L, $P < 0.001$), higher oestradiol levels (80.3 ± 27.8 vs 66.1 ± 29.0 pmol/L, $P < 0.05$), and higher ASI (204.5 ± 273.0 vs 96.2 ± 50.1 IU x nmol/L², $P < 0.001$). However individual hormonal data cannot distinguish men with and without AR gene mutations. Also clinical manifestations of AR mutations are not unique: two men had cryptorchidism, one had cryptorchidism and hypospadias, and one had gynecomastia, whereas 22 men had only spermatogenic impairment.

P0896. Screening for mutations in the GTPase mitofusin 2 that cause Charcot-Marie-Tooth neuropathy type 2A

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Charcot-Marie-Tooth neuropathy (CMT) comprises a genetically heterogeneous group of hereditary neuromuscular diseases with a similar clinical picture. Now some disease-causing genes have been identified for the axonal variant of autosomal dominant CMT or CMT2.

Recently the *MFN2* gene has been identified as the cause of Charcot-Marie-Tooth neuropathy 2A. Fibers of mitofusins, which reside at the outer mitochondrial membrane, determine the mitochondrial network architecture by fusion of mitochondria. *MFN2* is involved in apoptosis by colocalization with the proapoptotic protein Bax, and it is essential for embryonic development.

In 57 families with CMT2 and a classic clinical phenotype we undertook mutation screening by SSCP (single-strand conformation polymorphism); variants were characterized by bidirectional sequencing of the coding region of the *MFN2* gene.

In two families we found two new mutations Arg94Trp and Val705Ile in exons 4 and 18. In four families we found the previously described mutation Arg94Gln in the fourth exon. Results of our research confirm that mutations in the *MFN2* gene are the cause of CMT2A. The majority of the mutations were in exon four, related to the GTPase domain.

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P0897. Molecular consequences of *MTM1* mutations in X-linked myotubular myopathy muscle

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X-linked myotubular myopathy (XLMTM), caused by *MTM1* mutations, is one of the more severe congenital myopathies. Clinically, patients

present at birth with generalized weakness and hypotonia leading to respiratory distress, major feeding difficulties, and often death in infancy or early childhood. Pathologically, myofibers are hypotrophied and a high proportion has central nuclei. *MTM1* codes for a lipid phosphatase called myotubularin, but the molecular impact of its loss in XLMTM pathogenesis remains unknown.

Using Affymetrix whole genome arrays, skeletal muscle samples from 7 XLMTM patients (with *MTM1* mutations) versus 7 age-matched unaffected individuals, and 5 *Mtm1* knockout (KO) mice versus 5 wild type mice were analyzed. Data were processed by a combination of bioinformatic approaches including Significance Analysis of Microarrays (thresholds: fold \geq 2 and \leq 5% false discovery rate) and geometric fold change analysis.

The molecular consequences of *MTM1* mutations were mostly consistent across both species and involved over-expression of genes related to cell surface receptor linked- and intracellular-signalling, cell proliferation, muscle development, transcription and phosphate metabolism. Collectively, these data support alterations in extracellular signalling potentially affecting muscle function, a highly active transcriptional response and ongoing muscle regeneration. The numerous alterations observed at the protein synthesis, modification and more importantly at the vesicle transport level will enable a better characterization of the *MTM1* role in intracellular events such as endosome trafficking.

These data shed light on the molecular pathways involved in XLMTM pathogenesis and further validate the *Mtm1*-KO mouse as a disease model for future molecular and therapeutic target studies.

P0898. Mitochondrial disease in infant: clinical course and differential diagnostics. Case report.

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Girl at the age of 1 month was hospitalized due to the respiratory distress, failure to thrive and muscular hypotonia. After the birth persistant ketotic hypoglycaemia was reported during 1st week of life. Clinically she presented: respiratory distress, severe hypotonia, slightly dysmorphic phenotype with myopathic face. Biochemical analyses revealed progressive lactic acidosis (up to 9,1 mmol/l). In differential diagnoses several metabolic and genetic conditions were considered: galactosaemia, fatty acid oxidation defects, spinal muscular dystrophy, myotonic dystrophy, Prader-Willi syndrome, congenital hypopituitarism. Due to the clinical problems and progressive lactic acidosis mitochondrial myopathy was suspected and the diagnoses was confirmed by muscle biopsy.

Paper discusses differential diagnostic difficulties and the effect of supportive treatment to the course of mitochondrial myopathy in infant.

P0899. Mucopolysaccharidoses in Russia

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The mucopolysaccharidoses (MPS) in Russian population are on the decrease in the next order: MPSII -> MPSI -> MPSIII -> MPSVI -> MPSIV -> MPSVII. The DNA-diagnosis for MPSII (the iduronate-2-sulfatase gene), MPSI (the α -L-iduronidase gene) and MPSIIIA (the heparan-N-sulfatase gene) was developed. 62 patients with MPSII were investigated. Only 7% of mutant alleles detected were major structural alterations, other mutations were point mutations, small deletions and splice mutations. The known hot-spot mutations in IDS gene in codon 88, codon 274, codon 468 account for 7%, 7% and 10,5%, respectively. Twenty mutations found were novel ones. 55 patients with MPSI were analyzed for mutant alleles. The most frequent mutation in Russian population is Q70X. The Q70X accounts for 52,7 %, that is similar with results in Scandinavian population (62%). Both the W402X mutation and the Q380R mutation account for 4,6 %. Nine mutations were new ones. 13 patients with Sanfilippo type IIIA were investigated. The genotypes of six patients were fully determined. Two patients were homozygotes for C74R and two were genetic compounds C74R/K24H. One patient was homozygote for del1135G. Three mutations were found in sixth MPSIIIA patient: S73I, P230L and T139I. The novel

mutations S73I and P230L were inherited from the father. The proving their functional consequence on heparan-N-sulfatase activity must be studied. The polymorphisms R456H, ins5 c->t+17 and G198G in the heparan-N-sulfatase gene were also detected. Successful prenatal diagnostics for different types of MPS and carrier detection for MPSII was performed using DNA-analysis results.

P0900. MLPA technology: low cost, high throughput substitute for multiFISH technology in routine screening of telomeric rearrangements

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Cryptic telomeric rearrangements (CTR) are found in 5 % of patients with idiopathic mental retardation (MR) or Multiple Congenital Anomalies/MR syndromes, and apparently normal karyotype. Despite its clinical relevance, multiFISH screening remains difficult to apply widely, because kits are expensive and methodology extremely time consuming. Clinical preselection criteria as De Vries scale are unsatisfying.

Multiplex ligation-dependent probe amplification (MLPA) is a highly sensitive and rapid alternative to multiFISH. It can be used on any DNA source (blood, amniocytes, CVS, paraffin-embedded tissue...) We used 2 commercial sets of probes, the SALSA P019 combined to P020 and the P036 human telomere MLPA kits (MRC-Holland, Amsterdam, Netherlands) to assay retrospectively patients with known dup/del syndromes, and to test a group of more than 100 systematically collected patients referred for evaluation of MR or autism. In parallel, we performed multiFISH "a-la-carte" in those with evocative phenotype or familial history, in order to evaluate how often a CTR would have been missed using a non systematic, clinically driven practice. In all cases with abnormal/dubious MLPA, confirmatory FISHing was performed. Preliminary results are extremely convincing. Detailed results of this ongoing study will be presented.

Conclusion: subtelomeric MLPA screening can be done without preselection, and appears to be cost-effective, time saving, and clinically rewarding, as some of the positive patients would not have been selected on clinical grounds for multiFISH. Moreover, the drastic reduction in FISH-based screening demand allows busy diagnostic laboratories to re-allocate time to refining the cytogenetics/molecular definition of "positive" cases.

P0901. Molecular genetic analysis of X-linked severe combined immunodeficiency in two Russian families

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X-linked severe combined immunodeficiency (X-SCID) is a rare, life-threatening immune disorder, caused by mutations in the gene encoding the gamma subunit of the interleukin-2 receptor (IL2RG). The gamma-c chain is shared by 5 interleukin receptor complexes: IL2, IL4, IL7, IL9, and IL15. This disease characterized by a block in early T-, B-, NK-cell differentiation, agammaglobulinemia, lymphocytopenia, thymus atrophy, vulnerability to different infections and early death. IL2RG gene mapped to Xq13.1 contains 8 exons and 7 introns and spans approximately 4.2 kb.

We have found two different IL2RG gene mutations in the two patients with X-linked combined immunodeficiency. In the first case a novel 9-nucleotide insertion in 6 exon (823nt ins9bp) was detected by the direct sequencing analysis and PCR-RFLP. In the second case we found a unique splice site mutation (IVS5 as-2nt a->g) in 5 intron of IL2RG gene. Both patient's mothers were heterozygous for found mutations. The prenatal diagnosis of X-SCID and determination of heterozygous carrier for proband's maternal aunt were carried out for the first family. We have worked out the direct mutation analysis for the IL2RG gene and have identified two specific mutations in the families with X-SCID

P0902. Functional consequences of NSDHL mutations in CHILD syndrome

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Mutations in the gene NSDHL (NAD(P)H steroid dehydrogenase-like protein) encoding a 3β-hydroxy-steroid dehydrogenase functioning in the cholesterol biosynthetic pathway are associated with CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform Nevus and Limb Defects), an X-linked dominant, male-lethal trait characterized by an inflammatory nevus that usually shows striking lateralization with strict midline demarcation as well as ipsilateral hypoplasia of the body.

The phenotype appears to be caused by loss of function because it can be associated with nonsense- and missense mutations as well as with deletions eliminating several exons or the complete gene. Highly conserved amino acids of NSDHL located outside the predicted domains that are essential for the enzymatic activity (co-factor binding site, catalytically active site, transmembrane helix) may pinpoint positions of potential functional importance.

We generated by mutagenesis human NSDHL transgenes reflecting missense-mutations observed in CHILD patients or having altered other potentially functionally important sites. GFP-NSDHL fusion protein constructs with wild type or mutated NSDHL were transiently expressed in different cell lines. By associating the localization of GFP-NSDHL with cellular compartments (identified by immunohistochemistry) we demonstrate that the wild type protein primarily localises to the surface of lipid storage droplets (LDs) and to the ER. In contrast, expression of most of the mutant NSDHL variants results in altered subcellular organisation and disturbed localization of the protein.

Complementation analysis by transfer of mutated human NSDHL into the erg26ts yeast strain, which is mutated in the orthologous gene, ERG26, suggests functional differences between mutants, which are not reflected in the human phenotype.

P0903. Investigation for intragenic rearrangements of the *PLP1* gene in patients with dysmyelinating leukodystrophies using MAPH

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PLP1 is known to be mutated in the X-linked myelination disorders, Pelizaeus-Merzbacher Disease (PMD) and Spastic Paraplegia type 2 (SPG2). The most common *PLP1* mutation in PMD is a large duplication including the whole gene, whereas large deletions and punctual mutations can be responsible for PMD or SPG2 phenotypes. Semi quantitative PCR (only on 1 or 2 exons) is largely used to quantify *PLP1* gene copy number; looking only for large rearrangements. In the aim to search for small intragenic *PLP1* rearrangements in patients presenting classical forms of PMD or SPG and not diagnosed as *PLP1* mutated, we developed the MAPH (Multiplex Amplifiable Probe Hybridization) technique using 13 *PLP1* probes (2 promoter, 4 intronic and 7 exonic regions probes). This technique relies on the quantitative recovery and amplification of nucleic probes after hybridization to immobilized DNA.

In a first approach, DNAs from 66 patients and 22 carrier mothers already diagnosed with duplications have been used to validate the technique and have shown that all duplications encompass the entire *PLP1* gene.

Then, a total of 250 patients presenting with dysmyelinating leukodystrophies (including PMD or SPG like phenotypes) were screened. Whereas no duplications could be detected, two partial deletions of *PLP1* were identified. One deletion involves the 5' part of the gene (promoter to part of intron 1); the other one is a 3' gene deletion (exon 6 to 7).

MAPH is a powerful and cost effective tool to screen cohorts of patients for small intragenic or large genomic rearrangements.

P0904. Polyadenylation signal site mutation in a boy with X-linked Severe Combined Immunodeficiency

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The X-linked recessive form of Severe Combined Immunodeficiency (X-SCID) is characterised by a complete absence of both humoral and cell-mediated immunity and classically presents with no T cells or natural-killer (NK) cells. X-SCID is caused by defects in the common gamma chain of the interleukin -2,-4,-7,-9 and -15 receptors (IL2RG or IL2Ryc). The gene consists of eight coding exons and mutations are generally family specific.

We report on a 4 month old boy who presented with a clinical XSCID phenotype and abnormal gamma chain protein expression. However, following a sequencing screen of the eight exons and exon/intron boundaries no disease-causing mutation was identified. X-inactivation analysis in the proband's mother showed she was highly likely to be a carrier of an IL2RG gene defect. Linkage analysis using X-inactivation markers showed the proband had inherited the X-chromosome found to be inactive (methylated) in his mother's T cells. These factors increase the likelihood that an unidentified mutation affecting the IL2RG gene was responsible for the clinical phenotype. PCR analysis showed the genomic sequence was uninterrupted and that genomic rearrangement was unlikely. Promoter and intronic sequence analysis at Genome Research Limited, Hinxton, revealed a mutation in the polyadenylation signal site of the IL2RG gene. This case report has identified the limitations of exon screening in molecular diagnostics and has highlighted a mutation affecting primary RNA transcript processing which leads to a clinical phenotype.

P0905. Association of partial AZFc region deletions with spermatogenic impairment and male infertility

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Complete deletions of AZFc in distal Yq represent the most frequent molecular genetic cause of severe male infertility. They are caused by intrachromosomal homologous recombination between amplicons - large, nearly identical repeats- and are found in 5-10% of azoospermia and severe oligozoospermia. Homologous recombination may also generate different deletions of part of AZFc, but their contribution to spermatogenic impairment has not been confirmed. We analysed the prevalence and characteristics of different partial AZFc deletions and their association with spermatogenic failure. We studied 337 infertile men and 263 normozoospermic fertile men, by AZFc-specific STSs (allowing to detect partial deletions within AZFc) and DAZ-specific single-nucleotide variants (allowing to detect the DAZ gene copy number). We identified 18 cases of partial AZFc deletions in the infertile group (5.3%) and one case in the control group (0.4%). Seventeen deletions had the "gr/gr" pattern, one the "b2/b3" pattern, and one represented a novel deletion with breakpoints in b3 and b4 amplicons. Partial AZFc deletions were associated with different spermatogenic phenotype ranging from complete azoospermia to only moderate oligozoospermia. Analysis of DAZ gene copy suggested that the contribution of the different deletions to male infertility varies: only partial AZFc deletions removing DAZ1/DAZ2 seem to represent risk factor for male infertility, whereas deletions removing DAZ3/DAZ4 may have no or little effect on fertility. Although men with partial AZFc deletion may naturally conceive, in vitro fertilising techniques may transmit the mutation on to the male children and the reintroduction of these partial deletions into the population is of concern.

P0906. Screening for two susceptibility-associated polymorphisms of OCTN carnitine transporter in Crohn-disease patients

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Crohn-disease is a chronic inflammatory illness of the gastrointestinal tract. Previous data showed that, in addition to the well-known NOD

mutations, polymorphisms of the 5q31 chromosome region can be in connection with an elevated risk for the disease (Rioux et al., *Nat Genet* 2003;29:223). Recent studies indicated that allelic variants C1672T in the 9. exon of *SLC22A4* gene and G-207C in the promoter of the *SLC22A5* gene are susceptibility factors for Crohn-disease (Peltekova et al., *Nat Genet* 2004;36:471). The two rare alleles show a high linkage disequilibrium and make up a haplotype (TC) which was found to be significantly more frequent in patients than in the healthy controls. With direct sequencing of these two polymorphisms we investigated the prevalence of the haplotypes in a group of patients with Crohn-disease. Furthermore, we analyzed the carnitine-ester profile in the serum samples of these persons with ESI tandem mass spectrometry. Comparing the results of 53 patients and 50 healthy controls we found that the allele frequencies of the both polymorphisms significantly differ from those described in the above referenced original article and it was the case also for the linkage data. In the carnitine-ester profile of patients whose small intestine was affected a shift was detectable in the serum level of C2, C4, C5, C5:1 and C6 acil-carnitines when compared to controls. These results indicate that the carnitine system can be involved in the Crohn-disease primarily or secondarily.

P0907. Prevalent mutations in the *SURF1* gene of patients with Leigh Syndrome of Russian origin.

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Leigh syndrome (LS) is one of the most frequent forms of mitochondrial diseases in infancy and childhood. Typical presentations of LS are in the first year of life, with failure to thrive, psychomotor regression, ataxia, signs of brainstem dysfunction, and peripheral neuropathy. Etiologies of LS include both mitochondrial and nuclear DNA defects. Mutations in *SURF1* have been shown to be an important cause of LS with cytochrome c oxidase (COX) deficiency. The human *SURF1* gene encodes a protein localized in the inner mitochondrial membrane and is thought to be involved in the biogenesis of the cytochrome c oxidase complex. We found 4 different mutations in *SURF1* gene in 10 of our patients with LS. All of mutations have already been described: 312_321del311_312insAT, InsT868, DelCT845, insCTGC588. Spectrum of mutations in this gene is slightly different from other European patients in which mutation 312_321del311_312insAT is more common. In Russian patients with LS the most frequent mutation is 845delCT (accounts for 65% of mutant alleles) that is close to the frequency of this mutation found in Polish patients (66%). We found association between mutation 845delCT and SNP detected in the middle of intron 1 of *SURF1* gene (IVS1+56C>T). All patients presented both the 845delCT mutation and the IVS1+56C>T polymorphism. The SNP was absent in 50 control samples.

P0908. Recombination hotspot in the vicinity of the *SHOX* gene defines a common genetic cause for short stature

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Aside from the influence exerted by environmental and internal factors, growth is orchestrated by a large number of different genes. One of them, *SHOX*, is believed to play a major role since defects in this homeobox-containing short stature gene on the sex chromosomes lead to syndromal (Léri-Weill, Langer and Turner syndrome) or idiopathic short stature. We have analysed 118 independent patients with Léri-Weill dyschondrosteosis and 1,500 patients with idiopathic short stature for deletions encompassing *SHOX*. Deletions were detected in 34% of the patients with Léri-Weill dyschondrosteosis and 2% of the patients with idiopathic short stature. For 27 patients with Léri-Weill dyschondrosteosis and 6 with idiopathic short stature, detailed deletion mapping was carried out. Analysis was performed by PCR using pseudoautosomal polymorphic markers and fluorescence *in situ* hybridisation using cosmid clones. Here we show that the identified deletions vary in size, yet the vast majority of patients share a distinct deletion breakpoint. This breakpoint region is characterised by locus-

specific low copy repeats and a high preponderance of Alu and LTR sequences, which create a recurrent deletion breakpoint that occurs in more than 1% of all short individuals. It also represents one of the most frequent deletion breakpoints leading to disease.

P0909. Novel CAPN3 mutations identified in a routine diagnostic setting for LGMD patients

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Limb Girdle Muscular Dystrophies (LGMD) constitute a genetically and clinically heterogeneous group of autosomal dominant (LGMD1) or recessive (LGMD2) inheritance. LGMD2A is caused by mutations in the gene encoding Calpain-3 (*CAPN3*, 15q15.1-q21.1), a muscle-specific non-lysosomal protease.

LGMD2A is estimated to account for 10 to 30% of recessive LGMDs. It is the most prevalent form of LGMD and requires proper settings for diagnosis in affected patients.

As there is a high clinical variability of the phenotype, Calpain-3 protein analysis on muscle samples is performed at first instance to guide diagnosis. However, diagnosis has to be confirmed by molecular analysis of the *CAPN3* gene. The large size of the *CAPN3* gene (> 45kb, 24 exons) and the increasing number of reported allelic variants is technically challenging. Methods for mutation screening are particularly adapted to this task.

Here, we report a study of *CAPN3* mutations in a cohort of LGMD patients, identified using SSCP and/or DHPLC mutation screening, and subsequent sequencing of detected variants, in our clinical setting.

Using this strategy, at least one constitutive deleterious mutation was evidenced in 28 out of 39 included patients. A total of 45 mutations was identified, most of them being missense mutations (38%), or out of frame deletions and/or insertions with consequent frame shifting (31%). Among these mutations found in our series, 13 have not been reported before in the literature.

Diagnosis of LGMD2A could be confirmed in 17 out of 39 patients, by identifying two constitutive pathogenic mutations.

P0910. Functional characterization of 14 *SMPD1* mutations identified in Italian patients affected with NPD type B

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Niemann Pick disease (NPD) is an autosomal recessive lysosomal storage disorder caused by the deficient activity of acid sphingomyelinase due to mutations in the *SMPD1* gene. The disease has been divided into 2 different phenotypes: type A is a severe neurodegenerative disorder while type B is a non-neuronopathic disorder. However, the clinical course of type B NPD patients is quite heterogeneous. While some mildly affected patients can present a normal life-span several patients present with an intermediate phenotype characterized by mild neurological involvement. In the present study we characterized by transient expression in COS-1 cells three novel mutations, p.V130A, p.V557fsX18 and p.D563Y found in two NP type B patients and other 11 *SMPD1* mutations previously reported: p.M1_W32del, p.W32X, p.G34fsX42, p.L103P, p.P189fsX1, p.P192fsX14, p.L225P, p.W244C, p.A281T, p.R600H, p.R600P. Eight ASM mutants, p.W32X, p.G34fsX42, p.P189fsX1, p.P192fsX14, p.L225P, p.W244C, p.A281T, p.P557fsX18 had no significant enzyme activity. In contrast, mutations p.M1_W32del, p.L103P, p.V130A, p.D563Y, p.R600H, p.R600P expressed ASM enzymes with activities that range from 4.14 % to 28.4% of those obtained with wild type. Western blot analysis showed that p.L103P, p.V130A, p.L225P, p.W244C, p.A281T, p.D563Y, p.R600H and p.R600P mutants express ASM protein at levels comparable to that found in wild-type transfected cells. No immunoreactive protein was detected in cells transfected with p.M1_W32del, p.W32X, p.G34fsX42, p.P189fsX1 and p.P192fsX14 mutants. A smaller band was detected in cells transfected with p.V557fsX18 mutant, consistent with a presence of a premature termination codon. These findings provided valuable

insights into the molecular basis of the NPB phenotype variability.

P0911. Dominant LMNA or recessive ZMPSTE24 mutations lead to Restrictive Dermopathy, and nuclear accumulation of truncated or normal lamin A precursors

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Restrictive Dermopathy (RD) is characterized by intrauterine growth retardation, tight and rigid skin with prominent superficial vessels, bone mineralization defects, dysplastic clavicles, arthrogryposis and early neonatal death. In two patients affected with RD, we recently reported two different heterozygous splicing mutations in the LMNA gene, leading to the complete or, as commonly reported in Progeria, partial loss of exon 11. In other patients, a c.1085_1086insT insertion leading to a premature termination codon was identified in a T stretch in ZMPSTE24 encoding an endoprotease essential for Lamin A maturation. Known autosomal recessive inheritance of RD suggested a 2nd molecular defect was missing. We have explored ten RD patients; 8 of them are either homozygous or compound heterozygous for ZMPSTE24 mutations. All carry the c.1085_1086insT insertion as a common mutation, seven of them being homozygous for this defect although some were previously interpreted as heterozygous due to a sequence instability within the T stretch, leading to a pseudo-frameshift aspect. A large genomic deletion covering exons 3, 4 and 5, was also found as 2nd mutation in one case. Proteic studies showed complete loss of ZMPSTE24 and Lamin A expression, together with prelamin A expression and accumulation in all patients, including those for which the second ZMPSTE24 mutation is not yet identified. Thus, RD is a primary or secondary laminopathy, due to dominant de novo LMNA mutations or recessive ZMPSTE24 mutations most of them lying in a hotspot. The accumulation of truncated or normal length prelamin A is, however a common pathophysiological mechanism.

P0912. Cytochrome P450 CYP2C9 polymorphism in Turkish epileptic patients

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Cytochrome P450 CYP2C9 is a major enzyme that metabolize different clinically important drugs which include the anticonvulsant phenytoin. In this study the frequency of CYP2C9*2 and CYP2C9*3 allelic variants associated with phenytoin clearance were examined in Turkish epileptic patients who had received chronic phenytoin treatment. The patient group was selected according to the abnormal clinical response at usual dosage regimens and the allelic variants were studied by polymerase chain reaction and restriction fragment length polymorphism. The frequencies of CYP2C9 genotypes in the study group were 78.78%, 12.12%, 6.06%, 3.03% for CYP2C9*1/1, CYP2C9*1/2, CYP2C9*1/3 and CYP2C9*2/3 respectively. Although it is not statistically significant, all allelic variants found to be associated with reduced catalytic activity compared to wild-type with respect to mean phenytoin serum concentrations at 12h after dosage. 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.

P0913. Peculiarities of the primary structure of fibrillogenic proteins

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One of the proteins which can cause primary amyloidosis is transthyretin (TTR). Deposition of TTR fibrils in tissues leads to different pathological conditions such as familial polyneuropathy, cardiomyopathy and senile systemic amyloidosis. This work is devoted to the elucidation of the common principles of protein fibrillogenesis and to identifying the TTR molecular regions which are responsible for fibril formation. We analysed the primary structure of some fibrillogenic proteins and peptides: TTR, phage T4 lysozyme peptide (T4LP), peptide from A β (AP), human α lactalbumin (ALH) and ovalysozyme (LYZ) with a number of computer methods. We found that T4LP and AP have identical and similar aminoacid residues at an equal distance from one residue in both directions. Similar symmetry was also discovered on the C- terminus of the TTR sequence and in β -domains of LYZ and ALH. X-ray structure analysis of these proteins showed that residues before the symmetry center form β -sheet hydrogen bonds, however symmetrical residues don't take part in the β -sheet interaction, but are sterically able to form intermolecular hydrogen bonds. The most amyloidogenic recombinant mutant human TTR (L55P) was obtained from a bacterial system. We found that TTR variant which was purified without protease inhibitors does not support fibrillogenesis. We show that there was proteolysis of the terminal part of every TTR subunit. By contrast, TTR purified in the presence of protease inhibitors formed fibrils over several hours. We suppose that the "restricted" protein can't form fibrils under any conditions because of loss of the symmetrical part.

P0914. Screening for genetic alterations in PRNP gene of six different populations with determination of codon 129 genotype - a multicentre study

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The codon 129 genotype of PRNP gene is known to be a susceptibility factor for sCJD. Comparison of codon 129 genotypes reveals heterogeneity among different populations and can give some information concerning population patterns. We show data for six European populations.

DNA was extracted from blood of six groups: Germans, Slovenians, Czechs, Croatians, Macedonians and Bosnians and amplified PRNP coding region with PCR. Screening was performed with single stranded conformation analysis (SSCA). Samples, which formed SSCA patterns differently from majority, were sequenced.

We determined the proportion of alternative genotypes in each population.

Tested populations	M %	M/V %	V %
Macedonian	55,2	35,8	9,0
German	48,7	43,2	8,1
Slovenian	46,8	41,1	12,1
Czech	43,2	51,6	5,3
Bosnian	40,5	48,6	10,8
Croatian	38,5	47,7	13,8

Table: Percent of codon 129 genotypes in screened populations

We discovered 6 different SNPs and a 24 bp R3-4 type deletion.

Our results of codon 129 genotyping are not in accordance with already established east-west gradient. Croatian have lower and German higher proportion of M/M genotype than expected. Populations from former Yugoslavia differ in M/M more than in V/V genotype. Macedonian population differs from other screened populations from former Yugoslavia. Geographical distance, migrations concerning

work and former war in the Balkans caused mixing of populations to different extent. All cases of sCJD confirmed in our laboratory were methionine homozygote, suggesting high risk for population with high genotype M/M frequency.

P0915. The molecular genetic cause of osteopetrosis in Chuvashia

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Infantile malignant osteopetrosis is a rare autosomal recessive congenital disease characterised by failure of osteoclasts to resorb bones during their growth and development. The main features are severe anemia beginning in early infancy or in fetal life, hepatosplenomegaly, progressive blindness and deafness. Autosomal recessive osteopetrosis is genetically heterogeneous. All known genes are necessary for normal functioning of osteoclasts. One of the known genes is *TCIRG1*, which maps to 11q13.4-13.5. It encodes an osteoclast specific subunit of the vacuolar H⁺-ATPase proton pump. Our group investigated autosomal recessive osteopetrosis in 8 families from Chuvashia where the frequency of this disease is unusually high (1 affected per 3900 newborns by date of epidemiological study), probably due to a founder effect.

We have mapped this disease to the *TCIRG1* gene region (the most probable candidate gene) by analysing 4 polymorphic markers in this region. All affected probands are homozygous for all 4 markers and have an identical haplotype. All their healthy parents are heterozygous carriers for this haplotype; any other healthy relatives are not homozygous for this haplotype.

We then identified a new splice site mutation in *TCIRG1* by direct DNA sequencing of PCR-amplified exons and exon-intron junctions: all probands were homozygous and all parents were heterozygous.

By RFLP analysis we investigated the presence of the mutation in 327 healthy Chuvashians. The frequency of heterozygous carriers of the mutation was 3.4%.

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P0916. Tracking the roots of human mental retardation: cognitive impairments in Gdi1 knockout mice are associated with anomalous synaptic vesicles.

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X-linked non-specific mental retardation (XLMR) is a common genetic disorder characterized by mental handicap as the only clinical symptom. Among the genes identified is GDI1, which encodes one of the proteins controlling the activity of the small GTPases of the Rab family, involved in intracellular trafficking. A Gdi1 KO mice was investigated. Lack of Gdi1 altered availability and brain subcellular distribution of a subset of Rab GTPases (Rab3C, 4, 5 and 11) and caused hippocampus dependent deficits in short term memory formation. Electrophysiological analysis suggested depletion of the SV pool. Learning deficit due to impaired ability in CS-US pairing association in the trace fear conditioning test, was present when the inter trial intervals (ITI) between each pairing were short. By increasing the ITI, mutants were able to associate CS-US, further suggesting that mass training may eventually cause depletion of the SV pool, and that during a longer ITI, the KO mice could restore the SV pool for efficient memory formation and processing. Finally, EM analysis confirmed that the SV reserve pool was reduced in the KO, and showed that the defect was detectable at P10. In conclusion, our data demonstrated that lack of Gdi1 is responsible for alterations of synaptic vesicle biogenesis and for a number of direct and indirect changes in the hippocampus important for cognitive functions. Expression profiles of adult hippocampus of KO and WT mice were analyzed and a number of variations associated with the mutation and possibly involved in the cognitive impairments are under investigation.

P0917. Search for mitochondrial DNA T4,291C mutation in Hungarian metabolic syndrome patients

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Metabolic syndrome affects a very great percentage of the human population and is characterized by hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, hypomagnesemia, and obesity. In a recent study (Wilson et al, Science 2004;306:1190) hypercholesterolemia, hypomagnesemia and hypertension were shown to be transmitted on the maternal lineage in a large family. Homoplasic mitochondrial DNA (mtDNA) T4,291C transition was detected which ultimately led to the Ile-tRNA gene mutation characterized by replacing the uridine by cytidine immediately at the 5' position neighboring to the anticodon sequence. No data have been presented in the literature on the prevalence rate of this mutation. Using a simple PCR/RFLP assay developed for detection of the mtDNA variant coding for this mutation we genotyped 246 Hungarian patients with metabolic syndrome. We could not detect the pathologic mtDNA variant in any of our patients suggesting that this variant is not frequent in the Hungarian metabolic syndrome population, and routine screening is not recommended.

P0918. A second frame-shift mutation confirms the involvement of *MECP2* exon 1 in Rett syndrome

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Rett syndrome (RS) is a severe neurodevelopmental disorder affecting almost exclusively females and classically characterized by progressive loss of intellectual functioning, motor skills and language with development of stereotypic hand movements. RS is now recognized as one of the most common causes of severe mental retardation in females.

In our diagnostic laboratory we have analysed a consecutive series of 86 patients with clinical suspicion of RS by PCR-SSCA of *MECP2* exons 2, 3 and 4. Mutations were detected in 13/86 (15%).

Recently a new *MECP2* transcript comprising exons 1, 3 and 4 (MECP2B) was discovered (Mnatzakanian et al. 2004). The authors identified two exon 1 mutations in 19 typical RS patients with no known mutations in exons 2-4.

We reanalysed the 73 patients in whom no mutation had been identified in exons 2, 3 and 4, by direct sequence analysis of exon 1 and its flanking sequences. A single mutation was identified.

The patient, a 12-year old girl with RS, was heterozygous for an 8 base-pair deletion leading to a predicted premature stop codon at position 38; the mutation is coded *MECP2B* c.52_59delGAGGAGAG (p.Glu18_Arg20delfsX38). The mutation was *de novo*.

This mutation confirms the clinical significance of *MECP2* exon 1. We suggest that complete *MECP2* genetic diagnostic work-up must include exon 1 in both mutation and deletion screening.

P0919. Polymorphisms in the IL-4 and IL-4 receptor alpha chain genes and atopic bronchial asthma in North-West of Russia

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Background: Interleukin-4, a pleiotropic cytokine with immunomodulator functions, is involved in the upregulation of IgE production characteristic of atopic bronchial asthma (ABA). IL-4 by binding to its receptor (IL-4R) is essential for the development of airway inflammation present in asthma.

Objective: To investigate the role of two polymorphisms C-590T (IL-4) and Q576R (IL-4Ra) conferring susceptibility to the development of ABA.

Methods: The genetic polymorphisms of IL-4 and IL-4Ra chain genes were studied by PCR-RFLP analysis in two groups of Russian women: pregnant women with ABA (102 individuals) and healthy females (69).

Results: The distribution of genotypes and alleles of the Q576R polymorphism in the IL-4Ra gene were not significantly different between two studied groups. In contrast, we have found significant difference between patients and the control group in the distribution of genotypes for C-590T polymorphism of the IL-4 gene. The frequency of carriers - 590 T allele of IL-4 gene was significantly higher in asthma patients compared to control group (47% and 31% accordingly, $p < 0.05$; OR=1.9; 95% CI=1.0-3.6).

Conclusion: The results suggest, that polymorphism in the IL-4 might play a role conferring to susceptibility of asthma, and that IL-4Ra polymorphism is not associated with ABA in Russian population.

P0920. The results of molecular genetic analysis in Russian azoospermic and oligozoospermic men

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Many genetic factors had found to lead to male infertility. Besides chromosome aberrations major genetic causes of azoospermia and severe oligozoospermia are microdeletions of the Y chromosome (regions AZFa, AZFb, and AZFc) and CFTR gene mutations. We have examined 290 men with azoospermia and oligozoospermia diagnosed by sperm analysis. Molecular investigation was carried out on leucocyte DNA by analyzing SRY and ZFX/ZFY gene fragments and seven Yq-specific STSs: sY84, sY86, sY615, sY127, sY134, sY254, sY255 in multiplex PCR. Samples without deletions were analyzed for partial deletions of the AZFc region in another multiplex PCR, which included 6 STSs: sY1192, sY1197, sY1125, sY1206, sY1291, sY142. The group was also tested for 11 CFTR gene mutations (del21kb, delF508, delI507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821DelT, G542X, W1282X, N1303K) and IVS8 poly (T) variants.

AZF deletions have been found in 33 cases: 2 deletions in AZFa, 6 in AZFb+c, and 25 in the AZFc region. Five partial deletions of the AZFc region (del sY1192- 4 cases, del sY1197 - 1 case) have also been found in a group of 50 men without complete AZF deletions. The frequencies of Y-microdeletions in azoospermic and severe oligozoospermic men were about 12% and 8%, respectively. Six CFTR gene mutations in heterozygous state (del21kb/- - 3, delF508/- - 2 and W1282X/- - 1 case) were found in 73 men examined. Two infertile men were homozygous for the IVS8 5T variant and eight were heterozygous. In one case we identified a complete AZFc deletion and heterozygous IVS8 5T variant co-existing.

P0921. Simultaneous detection of the factor V Leiden, the prothrombin G20210A, the MTHFR C677T and the factor XIII Val34Leu variants by real-time PCR

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Real-time PCR melting curve-based genotype determination has become available for the determination of single nucleotide alterations. However, the growing variety of genetic alterations requires multiplexing of genotyping assays.

Here we report the simultaneous detection of the factor V Leiden, the prothrombin G20210A, the MTHFR C677T and the factor XIII Val34Leu variants by real-time PCR using a single tube four-color detection system.

One hundred and twenty six samples (including 24 quality controls) were blind-tested with this new assay and the results were compared to those obtained with a multiplex allele-specific PCR procedure usually performed in our laboratory.

Genomic DNA was extracted from 200 μ l EDTA-blood using an automatic procedure (MagNA Pure Compact) and eluted with 50 μ l of elution buffer of which 5 μ l were used per PCR reaction. Multiplex PCR reactions were set up in a final volume of 20 μ l containing the four primers and hybridization probe sets. Simultaneous mutation detection was achieved by analysis of the melting curves measured for each of the four sensor probes labeled with the LCRed 610, LCRed 640, LCRed 670 and LCRed705 fluorophores, specific for the MTHFR C677T, the factor V Leiden, the prothrombin G20210A and the factor XIII Val34Leu variants respectively.

The results were in perfect concordance with those previously obtained thus demonstrating the efficiency of the method for the simultaneous detection of single nucleotide polymorphisms. Combined with automatic

DNA extraction, it provides a rapid and convenient method to detect known mutations, dramatically reducing the total hands-on time.

P0922. Geno- and phenotype studies of myotonic dystrophy (DM1) in Hungarian patients

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Dystrophia myotonica type 1 (DM1) is a diffuse systemic disorder in which the most prominent features, i. e. myotonia and muscular atrophy may be accompanied by cataracta, gonadal atrophy, endocrine abnormalities, heart conduction defects and mental deficit. It is inherited as an autosomal dominant trait with a variable penetrance. An unstable expansion of (CTG)_n repeats in the 3' untranslated region encoding a member of the protein kinase family in 19q13.3 is the causative mutation for myotonic dystrophy. Healthy individuals harbour 5-37 CTG repeats, whereas in affected individuals repeat expansion varies between 37 and 4000. To examine the correlation between clinical expression and CTG trinucleotide repeat length, radioactive PCR as well as Southern blot analyses using probe p5B1.4 were carried out in families clinically diagnosed with myotonic dystrophy. So far, 28 patients from 14 families were analysed and in 11 cases the mutation in DMPK gene was confirmed. The expanded CTG repeats were transmitted maternally as well as paternally. In the maternally transmitted cases the expanded fragment lengths were always larger than in the paternally transmitted ones. Moreover, a clear correlation was established between phenotype severity and the length of the CTG expansion. Longer expansions resulted in earlier onset of the symptoms. Phenotypes varied between congenital onset, classical forms and mild symptoms even within the same family corresponding to the size of the expansion.

P0923. The X chromosome critical region for premature ovarian failure: alteration of chromatin organization around the translocations breakpoints.

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Monosomies and rearrangements of the X chromosome are often associated to premature ovarian failure (POF) and several independent studies have mapped a "critical region" for POF between Xq21 and Xq26. We have analyzed 25 X;autosome balanced translocations in Xq: 23 in patients and two in normal women. Considering all the breakpoints studied by us and by others we can exclude an effect of the chromosomal rearrangements per se and we tentatively exclude haploinsufficiency for Xq genes. The analysis suggested a position effect of the breakpoint on gene(s), X linked or autosomal, in the vicinity of the breakpoints. To investigate the hypothesis we have studied a region of 3 Mb in Xq21, where 8 breakpoints in patients and one in a normal woman were clustered and we mapped the X chromosome and two of the autosomal breakpoints. We studied the pattern of expression and the organization of the chromatin at the promoters and in flanking regions of the genes around the breakpoints. The chromatin organization appeared altered in breakpoint regions associated with a POF phenotype while it was unchanged in the normal woman. In situ hybridization analysis of the genes around the breakpoints in adult mouse showed that 5 genes in two autosomal breakpoints were specifically expressed at high levels in granulosa cells and/or oocytes. Alteration of the expression of these genes may be responsible of the POF phenotype in the patients.

P0924. Hereditary spastic paraparesis type 4 in Russian families

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Hereditary spastic paraparesia (HSP) type 4, or SPG4, caused by various mutations of spastin gene (locus 2p22) is the most common form in a heterogeneous group of autosomal dominant HSP's. In a recently begun molecular genetic study of HSP's in Russia, we have

so far detected three SPG4 families. Spastin mutation was found in one family with 4 patients in two generations. This frame-shift mutation 839-840delAG in exon 5 was previously described in a German family [Sauter et al., 2002]. Two unrelated families, one with 10 patients in five generations and another with 12 patients in four generations, showed linkage to SPG 4 locus, lod scores 1.66 and 1.51 correspondingly with recombination fraction equal to zero. Search of spastin mutation in these two and in a set of other HSP families is currently underway. As to clinical characteristics of the disease in three families, all patients (apart from one severe case presenting a mixture of HSP and hemolytic disease of the newborn) fall into relatively late-onset and slowly progressing "uncomplicated" HSP which is typical for SPG4; several persons, even elderly ones, have only subclinical signs of the disease. This work was support in part by President's RF grant MD-2456.2004.4

P0925. CFTR-opathies

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In the workshop "Lessons from CF", the importance of CFTR-opathies will be remembered. This importance has led to the latest international classification of CF together with its related diseases, when at least one CFTR gene mutation has been identified: CAVD, chronic pancreatitis, allergic bronchopulmonary aspergillosis, disseminated bronchiectasis, disseminated panbronchiolitis, sclerosing cholangitis, and neonatal hypertripsinemia. Still other diseases have been reported to have an increased frequency of CFTR gene mutations. In the case two mutations have been identified in an individual, generally at most one may be deemed causative of CF, while the other produces only a mild functional impairment and is not deemed to be causing CF disease. CFTR gene expression, including haplotypes and quantitative approaches to transcript analysis in different tissues, and modifier genes and environmental factors, modulate phenotypic expression in different CFTR-opathies. CFTR-related diseases may be thought of as extreme phenotypes of CF involving only one organ, or the result of modulation of gene expression in different tissues which may involve several organs even if in a minor degree. CFTR mutation screening is generally suggested for CBAVD and infertile couples before in vitro fertilization, while it is considered only in special cases (e.g. familiarity) for CFTR-related pulmonary or pancreatic diseases.

P0926. Model systems to study methylmalonic aciduria (MMA).

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The hereditary of methylmalonic acidemias are a devastating group of autosomal recessive metabolic disorders caused by defective isomerization of L-methylmalonyl-CoA to succinyl-CoA. In an attempt to develop tractable experimental systems to study this group of conditions, we have developed and characterized nematode and murine models. Genomic and biochemical characterization of the propionate to succinate conversion pathway in *C. elegans* was undertaken. Homologues of PCCA, PCCB, MMAA, MMAB, MMCM, and MMCR were identified in *C. elegans* and cloned for functional studies. Tracer experiments using C14-propionic acid and H3-phenylalanine revealed incorporation ratios similar to that seen in mammalian cells. The pathway could be stimulated by glucose and OH-cobalamin. Loading of *C. elegans* with propionic acid caused the animals to produce methylmalonic acid and accrete propionylcarnitine. A variable biochemical response to RNAi was seen. We also have created two new null alleles at the murine methylmalonyl-CoA mutase locus; one has exon 3 deleted and the 5' coding exon flanked by loxP sites, the other has a single loxP site in place of the 5' coding exon and both exons deleted. Both alleles should be useful for genomic manipulations such as Cre-mediated cassette exchange and Cre-mediated insertion. The mice display many features seen in the human condition, including massive elevation of MMA in body fluids, and perish shortly after birth. These diverse model organism systems will allow the human condition to be studied using modern genomic and genetic methods, and should facilitate the development and testing of new therapies for MMA.

P0927. Evaluation of a multiplex assay for cystic fibrosis using the Luminex platform

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Plans for newborn screening for cystic fibrosis (CF) and issues of sensitivity and efficiency with our current "home-brew" CF assay led us to look for a multiplexed CF assay which could be adapted to the mutation spectrum of the Irish population. We have an excellent knowledge of the Irish mutation spectrum, as all patients with mutations not detected by our ARMS assay are screened by DHPLC of the entire CFTR gene at the laboratory of Professor Claude Ferec in Brest, France. We evaluated the CF Multicode Plx(TM) assay from EraGen Biosciences. MultiCode uses an additional base pair constructed from the synthetic complementary bases isoguanosine (*isoG*) and 5'-Me-isocytosine (*isoC*). Base pairing of *isoG* to *isoC* is highly specific. These additional bases are used in each step of the MultiCode process: PCR, extension labelling, and liquid decoding on a Luminex instrument. All steps are carried out in the same micro plate well without transfers or washings.

We have taken the assay designed by EraGen and designed target-specific extension primers for 10 additional mutations which occur at a frequency of 0.15% or greater in the Irish population. We have evaluated the core 29-target assay and the extended assay on a large cohort of samples of known genotype, to examine their sensitivity and specificity. We are also evaluating a second Luminex-based assay, Signature CF(TM) from Ambion. Results of these studies, as well as an analysis of cost and efficiency, will be presented.

P0928. Characterization of *Caenorhabditis elegans* mutant strain RB839

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C. elegans is a widely used model organism. The aim of our ongoing study is to examine whether the genetically modified *C. elegans* strains may be suitable models for studying disorders of homocysteine metabolism. As deficiency of cystathione beta-synthase (CBS) is the main cause of homocystinuria in humans, we first started to characterize *C. elegans* strain RB839, in which a part of the F54A3.4 gene- an ortholog of human CBS- has been deleted.

Sequencing of genomic DNA obtained from RB839 revealed deletion between nucleotides 857 and 1548, which predicts an in-frame deletion of 231 amino acids in the putative active core of the enzyme. Subsequently we characterized the phenotype of RB839 nematodes. The body morphology, behaviour, lifespan and egg-laying of RB839 hermaphrodites did not differ from the wild-type N2 nematodes. We also determined the CBS activity in RB839 strain, which did not significantly differ from the control strain N2, suggesting that F54A3.4 gene does not serve as cystathione beta-synthase in *C. elegans*. This notion is also supported by the lack of difference in concentration of homocysteine and other aminothiols in crude extracts of RB839 and wild type N2 strains.

These preliminary data show that the strain RB839 with deleted part of F54A3.4 gene- an ortholog of human CBS- may not be a proper model for studying human CBS deficiency. However, our study suggests that the F54A3.4 gene may confer other functions in nematode sulphur metabolism.

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P0929. Two novel heterozygous TGFBR2 mutations in Marfan syndrome

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Marfan syndrome (MFS, MIM 154700) is an autosomal dominant disorder of connective tissue with cardiovascular, skeletal and ocular abnormalities, due to mutations in the FBN1 gene (15q21.1). A second type of the MFS (MIM 154705) is associated with a second locus, MFS2, at 3p25-p24.2. Identification of a 3p24.1 chromosomal breakpoint disrupting the TGFBR2 gene in a Japanese individual with MFS led to consider TGFBR2 as a gene underlying association with MFS at the MFS2 locus. Overall, four mutations of the TGFBR2 gene

have been identified to date in MFS with prominent cardiovascular phenotype (Mizuguchi T, et al, 2004).

The TGFB2 encodes the human transforming growth factor beta type II receptor (70/80 kDa). It belongs to the serine-threonine kinase family of cell surface receptors, which regulate many cellular processes including proliferation, cell cycle arrest, apoptosis, differentiation and formation of extracellular matrix.

We identified two novel heterozygous mutations (M425V, P525R) in two unrelated patients with MFS, who were proven not to be carriers of FBN1 gene mutations.

The M425V mutation was identified in a 27-year-old male patient whose father died suddenly at the age of 40 of an unknown cardiovascular cause. He had major cardiovascular (aortic dilatation) and skeletal signs plus a minor cardiac and ocular signs. The P525R was identified in 17-year-old girl. Her phenotype was characterised by aortic dilatation (she recently underwent preventive cardiac surgery), major skeletal signs, retinal degeneration.

Both mutations were absent in 50 healthy controls. We confirm TGFB2 gene as associated with MFS with major cardiovascular involvement

P0930. Mucopolysaccharidosis type I: Molecular studies of IDUA gene in Czech and Slovak populations

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During the last 25 years the diagnosis of mucopolysaccharidosis (MPS) was proved enzymatically in 89 patients from Czech and Slovak populations (15 mil.), including 18 mucopolysaccharidosis type I (MPS I) patients. Fourteen of the patients had the severe form of the disease (Hurler syndrome), two had the mild form (Scheie syndrome) and two had intermediate Hurler/Scheie phenotype.

All forms of MPS I are caused by the deficiency of a lysosomal hydrolase, alpha-L-iduronidase (IDUA, EC 3.2.1.76). The disease is inherited in autosomal recessive manner. We have analyzed the entire IDUA gene coding region sequence, exon-intron boundaries and a part of 5' and 3' UTR in 15 Czech and Slovak MPS I patients. Up to now we identified 19 mutated alleles: W402X (9/19), Q70X (7/19), c.1614delG (1/19), R628X (1/19) and c.1917-1926del (1/19). Two patients with severe phenotype were homozygous for the prevalent mutations, W402X and Q70X, respectively. Two patients with Scheie syndrome were compound heterozygotes for one of the prevalent mutations combined with a mutation in exon 14 (W402X/c.1917-1926del and Q70X/R628X).

We observed six known polymorphisms (A8, A20, Q33H, L118, A314, A361T). The Q33H, A8 and L118 were polymorphic in approximately 1/3 of the patient as well as control alleles. Preliminary results of haplotype analysis based on polymorphisms A8, A20 and Q33H showed that the alleles containing the mutation Q70X shared the same haplotype, suggesting a founder effect and a common ancestral origin.

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P0931. Deletion scanning of the CFTR gene using multiplex ligation-dependent probe amplification

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Large genomic deletions in the CFTR gene (also called ABCC7) may account for several unidentified mutant alleles in cystic fibrosis and other CFTR-related disorders. We have employed multiplex ligation-dependent probe amplification (MLPA) to screen for the presence of genomic CFTR deletions in 24 patients who had cystic fibrosis or obstructive azoospermia with only one mutation identified after sequencing of the whole coding region. In control samples, four out of five known genomic deletions were readily detected by MLPA. The mutation 134del120ins300 (also called CFTRdelle1) was missed by this method because the probe is located upstream of the deleted exon. Our subsequent MLPA screening uncovered a new deletion, CFTRdelle17(2.5kb), that had previously escaped detection by routine sequencing. Examination of the breakpoints showed that a highly

polymorphic dinucleotide repeat at the IVS17b(TA)n locus is implicated in the generation of this deletion, which leads to the loss of the exons 17a and 17b in two unrelated German cystic fibrosis patients in the heterozygous state. We conclude that MLPA is a sensitive and robust method for the rapid deletion scanning of the CFTR gene in cystic fibrosis samples with hitherto incomplete mutation detection.

P0932. Mutations in the DYSF gene: the importance of analysing DNA and RNA

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Mutations in the dysferlin gene (DYSF) cause different muscular dystrophy phenotypes including Limb Girdle Muscular Dystrophy 2B, Miyoshi Myopathy and Distal Anterior Compartment Myopathy. These disorders are characterized by autosomal recessive inheritance, adult onset and elevated levels of serum CK. The DYSF gene maps to chromosome 2p13, has 55 exons and encodes a protein of about 237 kDa that is located in the sarcolemma.

We have studied 32 patients from 20 families clinically diagnosed as MM, LGMD2B and DAT through: 1) analysis of dysferlin expression by immunohistochemistry in muscle biopsies and by Western blotting in CD14⁺ peripheral blood monocytes and, 2) screening for mutations in the DYSF gene by sequencing cDNA from monocytes, amplifying 14 fragments that cover the 55 exons. Finally, genomic DNA was studied to check mutations found in monocyte RNA.

Seventeen different mutations were identified: 7 missense, 6 frameshift and 4 nonsense. One of the mutations consist in a G>A transition at position 1924 of the genomic DNA, causing a G519R change in the polypeptide chain. RNA analysis revealed that this apparent missense mutation causes a deletion of 34 nucleotides in exon 18 causing a frameshift that produces a stop codon in exon 19. The deletion was probably induced by the activation of a cryptic splice site.

We consider that when considering a new mutation it is necessary to perform the analysis of both genomic DNA and RNA, including the analysis of normal controls to prove the pathogenicity of the mutation.

P0933. The use of real time PCR for detection of proteolipid protein-1 gene (PLP1) copy number in Pelizaeus-Merzbacher patients.

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Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive disorder of CNS myelination. More frequent classic form presents shortly after birth by nystagmus, psychomotor delay and hypotonia and later by spasticity and cerebellar ataxia. Rarer connatal type of PMD shows rapid progression and is fatal in infancy.

PMD is caused most frequently by duplication of the PLP1 gene located on Xq22, less frequently by PLP1 point mutations and very rarely by PLP1 deletion. Carrier women show usually no clinical signs.

Our aim was to distinguish samples with 1, 2 and 3 PLP1 copies using real time PCR and to define non-overlapping diagnostic ranges of $\Delta\Delta Ct$ values for these groups. We tested 40 gDNA samples which were previously analyzed by microsatellite markers analysis: healthy men (n=10) (1 PLP1 copy), PMD patients (n=9) (2 PLP1 copies), control group of healthy women (n=7) (2 PLP1 copies), carrier women (n=14) (3 PLP1 copies). Samples were run on ABI 7900 in multiplex reaction using primers and TaqMan probes for exon 3 of PLP1 and exon 12 of albumin gene, which was used as endogenous control. Results were processed using the relative quantitative comparative threshold cycle method ($\Delta\Delta Ct$).

The ranges of $\Delta\Delta Ct$ values are: < 1,3 for healthy men, 1,7 - 2,3 for PMD patients and healthy women, > 2,5 for carrier women.

Non overlapping ranges of $\Delta\Delta Ct$ values were detected for selected groups. Real time PCR can be employed in PMD testing.

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P0934. A novel mutation in the SCO2 gene in a patient with cytochrome c oxidase deficiency and a Werdnig-Hoffmann disease phenotype

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Disturbances of respiratory chain complexes, especially cytochrome c oxidase (COX) deficiency, represent a group of inherited disorders with clinical, biochemical and molecular heterogeneity, which usually manifest in early childhood and affects tissues with high energetic demand (brain, muscle, heart). Targeted treatment is not available and the prognosis for patients is unfavourable. For genetic counseling it is crucial to find the molecular background of the disease. In a group of 69 children with COX deficiency, mutations in SCO2 were found in 8 children. We present a 3-months old girl with a Werdnig-Hoffmann phenotype but negative on mutation screening of the SMN gene. A mitochondrial etiology of the disease was suspected after detection of hypertrophy of the interventricular septum. Biochemical analysis revealed increased levels of lactate in blood and CSF and analysis of respiratory chain complexes in isolated muscle mitochondria showed markedly decreased activity of COX. Mutation analysis of mtDNA was negative but sequencing of SCO2 revealed heterozygosity for the common mutation 1541G>A and a novel deletion 1518delA, which results in a frameshift and production of a truncated protein. To illustrate the possible effect of 1541G>A on the Sco2 protein, we present here a computer model of Sco2.

Conclusion: SCO2 mutations should be considered in patients with the phenotype of Werdnig-Hoffmann disease. Clarification of the molecular background of mitochondrial diseases may help in genetic counseling in affected families and it also enables prenatal diagnosis in families at risk with nuclear encoded defects.

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P0935. Respiratory and bioaminergic alterations in Mecp2-deficient mice, an experimental model for Rett syndrome

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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. Yet, the breathing irregularity has puzzled clinicians because of its state-dependency. Breathing is regular during sleep and can switch from highly irregular to regular even during wakefulness. Because breathing can be regular many clinicians believe that breathing problems are a consequence of disturbed neocortical rather than brainstem mechanisms.

To date, little is known when it comes to cellular explanations for any symptom of Rett Syndrome. We performed experiments on wild-type and Mecp2-deficient mice to understand the role of the Mecp2 gene in respiration and bioaminergic systems. We show 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. Severe respiratory disturbances are also evident in the isolated medullary respiratory rhythm generating network of Mecp2-/- mice. We propose that breathing irregularities in Rett patients are due to a disturbed aminergic control of the medullary respiratory network.

P0936. Presence of cell free fetal DNA in peripheral blood of patients with ectopic pregnancy

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Presence of cell free fetal DNA in peripheral blood of pregnant women is a well known phenomenon. The quantity of DNA is changing during the pregnancy, and seems to be different in normal and pathological pregnancies. The possible applications by detection, quantification and analysis of cell free fetal DNA in non-invasive prenatal diagnosis is the focus of the research. The aim of our study was to detect and to measure the quantity of fetal origin DNA in the peripheral blood of patients with ectopic pregnancy, and to compare with blood samples of women with normal pregnancies. Blood samples of the patients were collected before operative laparoscopy from ten patients with suspect ultrasound finding, positive pregnancy test and elevated serum hCG level. Quantitative real-time PCR analysis of the SRY region of Y chromosome was performed in order to detect and to measure the quantity of cell free fetal DNA. The diagnosis of ectopic pregnancy was verified by histological examination. SRY region was detected in eight histologically proven ectopic pregnancy cases. The mean gestational age was 9 weeks. Relative quantity of cell free fetal DNA was between 1.067E-04 and 3.986E-02. The results of our study suggest that cell free fetal DNA in the maternal circulation is detectable in cases of ectopic pregnancies. Comparison of the relative quantity of cell free fetal DNA, gestational week and hCG levels between ectopic and normal pregnancies might have importance in early diagnosis of ectopic pregnancy.

P0937. Mutational screening of the RPGR gene in Spanish XLRP families

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Retinitis pigmentosa (RP) is a degenerative disease of the retina, characterized by night blindness and visual field constriction. X-linked form of RP (XLRP; MIM # 268000) is the most severe type of RP because of its early onset and the rapid progression. Five XLRP loci have been mapped: RP2, RP3, RP6, RP23 and RP24. The RP3 locus on Xp21.1 accounts for 60-90% of XLRP.

We have analyzed the RPGR gene (RP3 locus) in 34 unrelated Spanish XLRP families. We have performed haplotype analyses to assign the locus responsible of the pathology in each family, in order to determine if the disease is linked to RP3 region. In those families in which the disease segregates with the RP3 locus we have carried out mutational screening of the RPGR gene. We have analyzed the first 15 exons of RPGR at mRNA level and the ORF 14 and ORF 15 exons at DNA level by SSCP-PCR and automatic sequence.

After haplotype analysis, we were able to rule out the RP3 locus in 5 XLRP families (14,7%). In the rest, we have identified 15/29 RPGR mutated families, 8 of them were novel and affected individual families. The 2 previously described mutations g.ORF15 481-482 Del AG and g.ORF15 652-653 Del AG have been identified in 4 and 3 families respectively. We confirmed that exon ORF 15 is a mutational hot spot because 86,7% of the mutations found in our XLRP families were located in that region.

P0938. Evidence from autoimmune thyroiditis of skewed X-chromosome inactivation in female predisposition to autoimmunity

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The etiologic factors in the development of autoimmune thyroiditis is not fully understood. The disease is approximately fifty times more frequent in women than in men. The role of X-chromosome inactivation has been questioned in female predisposition to autoimmunity. Until now this has not been illustrated experimentally. We tested the hypothesis that disturbances in X-chromosome inactivation (XCI) mosaicism may be involved in the pathogenesis of autoimmune thyroid diseases (AITDs) Hashimoto thyroiditis and Graves disease. One hundred and nine female autoimmune thyroiditis patients and 160 female controls

were analyzed for the androgen receptor locus by the *Hpal*I/polymerase chain reaction assay to assess XCI patterns in DNA extracted from peripheral-blood cells. Furthermore, thyroid biopsy samples were obtained from five patients whose blood revealed an extremely skewed pattern of XCI, and the analysis repeated. Skewed XCI was observed in DNA from peripheral-blood cells in 27 of 82 informative patients (33 percent) as compared with 10 of 124 informative controls (8 percent, $P<0.0001$). Extreme skewing was present in 21 patients (26 percent), and only in three controls (2.4 percent, $P<0.0001$). However, XCI was random in all thyroid biopsy samples. Skewed XCI mosaicism may play a significant role in the pathogenesis of autoimmune thyroid diseases. Supported by grants from the Scientific and Technical Research Council of Turkey - TUBITAK-SBAG 2513, and International Center for Genetic Engineering and Biotechnology - ICGEB-CRP/TUR04-01.

P0939. Allelic series of arrhythmic disorders caused by different mutations in *SCN5A* gene.

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Molecular medicine seeks to explain why a particular mutation causes a particular clinical phenotype. The *SCN5A* gene has been implicated in a wide spectrum of inherited arrhythmic disorders. To date, at least six human diseases have been linked to mutations in the *SCN5A* gene: long QT syndrome type3 (LQT3), Brugada syndrome (BS), sick sinus syndrome (SSS), cardiac conduction defect (CCD), sudden infant death syndrome (SIDS), and idiopathic ventricular fibrillation (IVF). Here we present the results of genetic screening of the *SCN5A* gene. We screened 22 unrelated families with ventricular arrhythmias: 14 probands had LQTS, 6 had BS and 2 had IVF. All patients and their relatives had a detailed clinical checkup. Genomic DNA was isolated from EDTA venous blood by standard methods. For mutation screening, original intronic primers were developed that encompassed the complete coding sequence, the splice sites, and the adjacent areas. We found five *SCN5A* mutations in six probands: A572D and Q1033R in LQTS patients, 848del and IVS17DS-5A/G in a BS patient, P2005A in an IVF patient, compound mutations A572D and S1431R in a patient with a mixed phenotype (BS + SSS).

Different *SCN5A* mutations can produce different clinical phenotypes due to specific alterations of the inward I_{Na} current. Interestingly, the mutation A572D caused different clinical phenotypes in two unrelated patients: LQTS and BS. We suggest that this is due to the influence of other modifiers in *SCN5A* or other relevant genes (for example, these patients have differences in the SNPs distribution in *SCN5A*).

P0940. Extremely skewed X-chromosome inactivation is increased in preeclampsia

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Preeclampsia is a disorder that affects about 5% of the pregnancies in Europe and US. Major diagnostic criteria include gestational blood pressure increase without a history of hypertension before pregnancy and proteinuria. We tested the hypothesis that skewed X chromosome inactivation (XCI) could be involved in the pathogenesis of preeclampsia. Peripheral blood DNA was obtained from 55 preeclampsia patients and 131 age matched control women. Age, pregnancy history, blood pressure, and disease information were collected for clinical characterization of the patients. The androgen receptor locus was analyzed by the *Hpal*I/polymerase chain reaction (PCR) assay to assess XCI patterns in the DNA samples. Male DNA with cytogenetically verified 46, XY karyotype was used as control for complete digestion. PCR products were run on 10%PAGE and stained with ethidium bromide. XCI patterns were also quantitated by use of radioactive (α -[³³P] - dCTP (NEN) PCR. Densitometric analysis of the alleles was performed using the Multi-Analyst software version 1.1 (Bio-Rad Laboratories). Skewed XCI was observed in 13 of 38 informative

patients (34.2 percent), and in 10 of 124 informative controls (8 percent, $p<0.0001$; χ^2 test). Extreme skewing was observed in eight (21 percent) patients and three controls (2.4 percent, $p<0.0001$). These results raise the possibility that extremely skewed X-inactivation may have a role in the pathogenesis of preeclampsia.

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P0941. Molecular characterization of hemophilia A in the Republic of Macedonia

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Hemophilia A is a common X-linked bleeding disorder affecting 1 in 5,000 males worldwide. The most common molecular defect is intron 22 inversion, which participates with 45% of all mutations in the factor VIII gene. The rest of the mutations are caused by single nucleotide substitutions in different parts of the gene. The knowledge of the causative gene defect has become an important instrument in hemophilia care with respect to prediction of the patients' clinical course and safe genetic counseling of relatives. The aim of this study was to determine the molecular defects underlying hemophilia A patients from the Republic of Macedonia. The molecular characterization of fVIII gene was performed in 50 unrelated hemophilia A patients. Southern blot analysis was used for detection of inversions in intron 22, while PCR followed by SSCP or DGGE were performed for mutation screening. The molecular defect was found in 34, or 68%, of analyzed patients. The most frequent molecular defect was inversion in intron 22, found in 38% of all studied patients, or in 67.7% severely affected patients. Nucleotide substitutions were found in 15 (30%) patients. With the exception of C-T change in codon 2159 found in 4 (8%) of unrelated patients, the other nucleotide substitutions were private. Two of the mutations were nonsense (Cd272G-T, and Cd1696C-T) while eight were missense of which four were found for the first time (Cd-19Met-A; Cd78Ala-Pro; Cd2174Cys-Gly; Cd2256Tyr-Asp). Six prenatal diagnosis were successfully performed during this study.

P0942. Analysis of mutations in the *ABCB4* (MDR3) gene in Czech patients with cholestatic liver disease

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The *ABCB4* (MDR3) is a phospholipid export pump located predominantly at the biliary pole of the hepatocyte. Mutations in the *ABCB4* gene were found in homozygosity or heterozygosity in patients with progressive familial intrahepatic cholestasis (PFIC), cholesterol gallstones and intrahepatic cholestasis of pregnancy (ICP).

The *ABCB4* gene is located at chromosome 7q21.11 and spans more than 75 kb.

We have analysed 27 protein-encoding exons of *ABCB4* gene in 6 Czech families with cholestatic disease, familiar occurrence of cholesterol gallstones and recurrent contraceptive pill-induced cholestasis. In three of the families the probands carried on one of the alleles a mutation - c.3608C>G (S1203X), c.1501G>T (E501X), and c.1954A>G (R652G), respectively, and wild type sequence on the other. The fourth proband carried mutations c.523A>G (T175A) and c.1371del G (G457X).

In two of the families no apparently pathogenic mutations were found. The family 5 proband was heterozygous for 3 synonymous mutations (c.175C>T, L59L; c.504T>C, N168N; c.711A>T, I237I) and the sixth proband carried on one of the alleles the IVS28-16C>T, that is likely not pathogenic.

Our findings confirm that mutation analysis of *ABCB4* should be considered in the broadening spectrum of liver diseases including contraceptive pill-induced cholestasis and early onset familial

gallstones.

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P0943. Heterozygosity of eight STR markers studied by QF-PCR in spanish population

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QF-PCR is a recent method for rapid detection of chromosome dosage based on the analysis of highly polymorphic STR markers. These markers must have a large number of alleles with a low frequency to give enough information to discriminate the presence of aneuploidies. The application of this technique in the prenatal diagnosis field had permitted the diagnosis of the most common aneuploidies in less than 24 hours, reducing parental anxiety. However it is important to assess the heterozygosity of these markers for a reliable diagnosis.

In this study we have used eight markers for chromosomes 13, 18, 21 X and Y that have been previously described as highly polymorphic (see Table).

We have analysed a total of 172 samples. PIC (Polymorphism Information Content) has been calculated to ascertain if the markers were as informative in our population as previously described for other populations.

The results obtained show that these markers are very polymorphic (PIC > 0,7) although we found slight differences in the heterozygosity values previously described.

CONCLUSION: The markers previously described are polymorphic enough in our population, so they can be used for the study of the most common aneuploidies.

Marker	Chromosome location	Number of Homozygotes	Allele Range	Number of Alleles	PIC
D13S631	13q32.2	33/150	188-212	7	0.76
D13S634	13q14.13	30/114	462-496	9	0.8
D18S535	18q12.3	42/172	127-151	7	0.76
D18S386	18q22.1-22.2	9/111	318-402	21	0.91
D21S1414	21q21.1	38/156	326-366	11	0.82
D21S1411	21q22.3	25/150	267-315	13	0.88
X22	Xq28/Yqter	17/131	196-244	12	0.82
XHPRT	Xq26.1	18/154	273-297	7	0.72

P0944. Activated exonic splicing enhancer as a cause of homocystinuria

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Exonic splicing enhancers (ESE) are elements that interact with SR proteins and promote utilization of splice sites. Alteration of their motifs by mutations results in aberrant splicing. The aberrant splicing is also one of the causes of homocystinuria due to the methionine synthase reductase (MTRR) deficiency. In this case an intronic substitution T>C (IVS6+469T>C) of the *MTRR* gene putatively leads to activation of ESE and induces an insertion of 140 bp pseudoexon between exon 6 and 7. The aim of our study was to test the pathogenicity of this mutation.

The *in silico* analysis of the pseudoexon strongly suggests the proposal mechanism of action of the intronic mutation. The T>C substitution forms a new ESE motif for binding of the SR protein SF2/ASF. This ESE may provide recognition of the cryptic sites delimiting the pseudoexon.

Aberrant splicing was functionally studied *in vivo* using exon trapping technique. COS-7 cells were transfected with pSPL3 constructs carrying either the wild type or mutant pseudoexon. Splicing products

were analysed by RT-PCR with vector specific primers. The results clearly showed that the pseudoexon is recognized and inserted into mRNA only in the presence of the intronic mutation.

In conclusion, the *in silico* and functional analyses confirmed that the mutation in intron 6 causes aberrant splicing of *MTRR* transcript. Our study demonstrates that knowledge of general mechanisms of ESE action may contribute to the understanding of human genetic diseases.

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P0945. Novel germline mutations in the adenomatous polyposis coli gene in Polish FAP patients

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Familial adenomatous polyposis (FAP) is an autosomal dominant predisposition to initiate numerous polyps in the colon and rectum which develop to the carcinoma if left untreated. FAP is caused by inherited or germ line mutations in the *APC* gene. Early recognition of the mutation carriers is very important for the medical treatment of persons from the high-risk group. It is estimated that few hundreds of Polish FAP families will be subjected to genetic testing. The DNA bank for Polish FAP patients at the Institute of Human Genetics at Poznan was established in 1997. FAP diagnoses were established in the cooperating health centers. 554 DNA samples from persons belonging to 220 FAP families were collected. 248 patients were diagnosed with FAP, 16 with attenuated FAP and 10 with the Gardner syndrome. 215 persons belong to risk group and 67 persons do not belong to risk group. The entire *APC* gene coding sequence was screened for mutations in 195 families. Forty-five types of mutations were identified in 74 Polish FAP families. Twenty-nine of them have not been described before. Seven mutations types recurred two or more times. The recurrent mutations were detected in 52% of diagnosed families. From diagnostic point of view it was possible to diagnose almost all FAP cases on molecular level.

P0946. Identification of a deletion in chromosome 1, including the ABCA4 gene region, resulting in a Stargardt disease phenotype.

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INTRODUCTION: 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, mapped to 1p21-p13.

PATIENTS AND METHODS: A total of 85 STGD families were studied. DNA from the patient and relatives was analysed for variants in all 50 exons of the ABCA4 gene by screening on the ABCR400 microarray; the results were confirmed by direct sequencing. Haplotype analyses and a HR karyotype were also performed.

RESULTS: In one family segregating STGD, we found a patient with hemizygosity for a paternal missense mutation. By haplotype analyses, a maternal non-contribution with apparent segregation of a null allele was identified. Microsatellite markers spanning over 12 Mb could identify a microdeletion, of at least 7.5 Mb, involving the ABCA4 gene region. The cytogenetic study using high resolution techniques (800-900 bands) did not reveal this rearrangement.

CONCLUSIONS: The present study suggests that genomic alterations contribute to only a small fraction of disease-associated alleles of ABCA4. For those cases where a homozygous mutation is found, is recommendable to perform haplotype analyses in order to discard a possible situation of hemizygosity due to deletions.

P0947. Familial juvenile hyperuricemic nephropathy (FJHN), molecular analysis of 23 families, identification and functional consequences of 6 uromodulin mutations

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FJHN is an autosomal dominant renal disease characterized by juvenile onset of hyperuricemia, gouty arthritis, and progressive renal failure at an early age. In our study of 23 families we found linkage of FJHN to chromosome 16 (CH16) in 8 families. Mutations in the uromodulin (UMOD) gene (16p11.2) were recently proved to be a cause of FJHN. Analyzing UMOD coding sequence including exon/intron boundaries in all families, we found FJHN causing mutations (Cys32Tyr, Cys126Arg, Met229Arg, Pro236Leu, Val 273Phe and Cys317Tyr) only in 6 families.

To characterize pathogenic effect, we cloned identified mutations into eucaryotic expression vector and expressed them in AtT20 cells (not expressing UMOD). We investigated UMOD localisation (immunocytochemistry), post-translational modification (Western blot) and dynamics of plasma membrane exposition (flow cytometry).

Colocalization immunocytochemical studies proved storage of mutant proteins in endoplasmic reticulum. Western blot analyses revealed that mutant proteins varied in the extent of glycosylation and also in the relative distribution of the glycoforms. Flow cytometry showed lower level of exposed UMOD on plasma membrane in mutant proteins than in the wild type.

The results suggest two different mechanisms of uromodulin dynamics impairment in FJHN - retention in ER and impairment of protein exposition on plasma membrane.

P0948. Experience of molecular diagnostics for genetic disorders in Latvia

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The clinical application of genetic investigation started in Latvia in the Riga Medicine Institute, Mother and Children Protection Scientific Research Department in 1972. The Latvian State Medical Genetics Centre was organized as the only state medical genetics service in the Latvia in 1986.

DNA diagnostics started in 1997 with the most common autosomal recessive metabolic disorder as phenylketonuria.

Today DNA testing is available for 9 different genetic disorders: phenylketonuria, spinal muscular atrophy (SMA), X-linked Duchenne muscular dystrophy (DMD), medium chain acyl-CoA dehydrogenase deficiency, long chain hydroxyacyl-CoA dehydrogenase deficiency, nonsyndromic sensorineural hearing loss, Charcot-Marie-Tooth disease (CMT), Fragile X, and Y chromosome AZF deletions.

The following methods are used for DNA testing: PCR, multiplex PCR, restriction enzyme digestion, denaturing gel gradient electrophoresis, fluorescent PCR and PCR fragment analysis on ABI Prism 310, direct DNA sequencing.

Characterization of the molecular basis of PKU in Latvia has been accomplished through the analysis of 110 unrelated chromosomes from 56 Latvian PKU patients. The most frequent mutation, R408W, accounts for 78 % of Latvian PKU alleles, and six mutations (R408W, E280K, R158Q, A104D, R261Q and P281L) represent about 93 % of PKU chromosomes.

Fifty patients suspected of having spinal muscular atrophy were analysed. Fourteen patients (28%) are homozygous for the SMN1 deletion. Three patients have a confirmed diagnosis of DMD. Eight patients from 13 referred for molecular diagnostic have a confirmed diagnosis of CMT.

This year we are going to start DNA testing for Huntington disease and BCR-ABL gene transcript detection.

P0949. Use of multiplex SNaPshot reaction for the detection of five prevalent mutations in GJB2 gene in Czech prelingual deafness patients.

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Summary: Biallelic pathogenic mutations in GJB2 gene are detectable in 39,2% Czech patients with prelingual deafness. The 3 most common mutations (35delG, W24X, 313del14) represent 96,2% of all pathogenic mutations detected in the coding region of GJB2. Testing for mutation in intron1 of GJB2 rises the detection rate of GJB2 mutations. So far GJB2 mutations are tested by direct sequencing of the entire coding region at the exon2 and noncoding exon1. In 51,9% patients no mutation is found by sequencing. We evaluated a new method for simultaneous detection of the 5 most common GJB2 mutations (35delG, W24X, 313del14, delE120, IVS 1+1 G in A), which could make the DNA testing of GJB2 in hearing impairment patients faster and more simple. SnaPshot reaction represents a single-base extension reaction using only dye-terminator nucleotides. Products are analyzed on capillary electrophoresis analyzer ABI310.

Method: We tested 50 DNA samples from Czech patients with prelingual deafness which were previously tested by direct sequencing. 2 PCR fragments including both the coding exon2 and the noncoding exon1 of the GJB2 gene were tested in 2 SNaPshot reactions. The products of both reactions were analyzed simultaneously.

Results: Genotypes of all patients tested with SNaPshot were in correlation with the results of previous direct sequencing analysis.

Conclusion: Correct genotype of the GJB2 gene was detected in all 50 cases by multiplex SnaPshot. This method is suitable for further diagnostic procedure.

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P0950. Clinical Performance Validation Of A Diagnostic Kit For Common European Cystic Fibrosis Mutations

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Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. We have recently launched a new diagnostic device (kit) for genotyping mutations in the CF gene that meets the CE mark requirements of the European In Vitro Diagnostics Directive (IVDD). The kit is based on multiplex PCR amplification and subsequent probing of the various alleles by the oligonucleotide ligation assay (OLA). The assay detects the mutant and normal alleles for 33 common multi-ethnic mutations in a core panel. In addition, reflex reagents are provided for the genotyping of polymorphisms 5T, 7T, and 9T in intron 8 as well as for I506V, I507V and F508C in exon 10. Samples are automatically electrophoresed on the Applied Biosystems ABI PRISM® 3100 Genetic Analyzer. The resulting data are analyzed in an automated fashion using Applied Biosystems GeneMapper™ software and a template that has been configured for the CF assay. We demonstrated the clinical performance of the kit reagents and the CF-specific analysis system in a formal clinical trial using 3 external test sites and one internal site. Three lots of reagents were tested with a double-blinded panel of samples that contained all mutations to be detected by the assay. All samples were genotyped correctly by all four test sites demonstrating 100 % specificity and 100 % sensitivity.

P0951. Prenatal diagnosis in a spanish family affected with CMT1-A disease

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Charcot-Marie-Tooth disease is the most frequent inherited peripheral neuropathy. A microduplication of 1.5 Mb containing the gene for peripheral myelin protein 22 (PMP22) on chromosome 17p11.2 is responsible for the 75% of cases of demyelinating form (CMT1A). Diagnosis in our laboratory is performed with STR markers located inside the microduplication, according to the European Guidelines of

EMQN. The results are compatible with the diagnosis of CMT type 1 disease if we detect three alleles or if we detect dosage effect in at least two markers.

Prenatal diagnosis requires some special considerations: The mutation of the affected parent should be known, and it is also recommended to know the phase of the markers for both parents to facilitate the analysis.

We present the first prenatal diagnosis of CMT1A disease performed in our laboratory.

Two sets of PCRs with 8 STR markers were performed to construct the haplotypes of a family with an affected pregnant patient, her affected sister and her husband.

Four markers were informative (D17S839; D17S955; D17S1357; D17S921). We predicted the four possible embryonic haplotypes (two affected and two healthy). Peak patterns of the STR markers and the dosage effect was also estimated previous to the prenatal analysis, which ultimately showed that the fetus inherited the haplotype with the associated microduplication.

Conclusions:

1st The prenatal diagnosis of this pathology requires a molecular study of the couple previous to the prenatal study.

2nd Experience in STR genotyping methods for the detection of dosage effect is highly recommended.

P0952. Detection of two independent deletions of the SMN1 gene in the same SMA family branch: a word of caution for carrier diagnosis.

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Spinal muscular atrophy (SMA) is a common autosomal recessive disorder caused by mutations in the SMN1 gene. Relatives of affected patients usually request genetic counselling resulting in a high demand for carrier testing. A number of reliable quantitative methods for carrier detection have been implemented with a risk of approximately 5% for false negative results owing to the presence of two copies of SMN1 in the same chromosome. DNA markers linked to the SMN locus can be used to exclude carrier status and to complement the quantitative results.

We present a family with a SMA patient with homozygous deletion in the SMN1 gene in which 33 members of the paternal branch were analysed. Following Real-Time quantitative analysis, several first and second degree branch relatives showed one copy of the SMN1 gene and were diagnosed as carriers. Marker analysis in some members with one copy demonstrated discordance, being carriers of a deletion on a different chromosomal background. We concluded that two deletions of different origins exist in this branch of the family. Marker analysis allowed us also to identify carriers with two SMN1 copies in the same chromosome and one of the deletions in the remaining chromosome. In the light of the finding of two independent deletions in the same branch of the family carrier testing in SMA should be performed employing both, quantitative and marker analysis in order to avoid pitfalls in carrier identification and to allow a more accurate genetic counselling.

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P0953. Tess (Telencephalic Embryonic Subtractive Sequences): a sub-collection of genes potentially involved with Holoprosencephaly

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One of the neurodevelopmental defects for which several genes are still unknown is Holoprosencephaly (HPE), a birth defect involving the failure of the prosencephalon to cleave into the cerebral and lateral hemispheres. The aim of this project is the functional characterization of new genes involved in the developing mouse telencephalon as being potentially involved in human neurodevelopmental disorders

and correlated to HPE.

We used a bioinformatic analysis and annotation, a high-resolution expression analyses by mRNA in situ hybridization, together with a gene array expression experiments in both Neural Stem Cells and Neuro2A cells, to identify several "unknown function" genes expressed during the neural differentiation processes. Of the cDNA collection we studied 832 cDNA, of which 323 (39%) correspond to novel rare transcripts, and includes 48 (14%) new putative MicroRNAs. Because this selected enriched cDNA source contains genes expressed in telencephalon (between E.7.5 to E.14.5), we think this source is enriched for genes involved in telencephalon development, which may be responsible for HPE in humans. On the basis of their homology and map positions near human holoprosencephaly candidate regions, twenty-three human TESS homologues (two hypothetical pre-miRNA and twenty-one novel transcripts) were selected for further characterization. We will present results on their expression at early stages of mouse development (E. 7.5) by Real Time PCR. Then we will attempt to select the best candidates for mutational analyses in the DNA of patients affected with HPE. Additionally, two pre-miRNAs will be presented to identify and validate their targets as potentially involved in HPE.

P0954. Nine novel mutations in EYA1 and one novel mutation in SIX1 in a BOR patient cohort

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Branchio-oto-renal (BOR) syndrome is an autosomal dominant disorder, affecting both the internal and external ears, the branchial structures and the kidneys. It is estimated that in 50% of BOR patients, the genetic defect lies within the *Eya1* gene. Recently, *Six1* mutations were shown to account for a subset of *Eya1* positive BOR patients. *Eya1* and *Six1* physically interact and form an active transcriptional complex within the *Eya-Six-Pax-Dachshund* genetic network.

Over the last 5 years, we collected 55 BOR patients. In those patients, we identified three described and nine novel *Eya1* mutations. Ten mutations were identified in patients with a positive Family history, 2 additional mutations in sporadic cases. In addition, we tested six clearly diagnosed BOR patients for *Six1* mutations, and identified one novel missense mutation within the Six-domain.

In a substantial part of our BOR patient cohort, no causative mutation was found. Completion of *Six1* analysis may lead to the identification of additional causative alterations. Furthermore, gross deletions of the *Eya1* and *Six1* genes may be present in patients with no reported mutation. Another possibility is that several of our patients were misdiagnosed. However, it appears equally likely that mutations in novel BOR genes are responsible for the onset of disease.

P0955. Suspected triallelism in Leber Congenital Amaurosis detected using a genotyping microarray

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Leber Congenital Amaurosis (LCA) is the earliest and most severe form of all genetic retinal dystrophy causing blindness. 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 of the retina.

We report a mutational analysis of all eight genes in 111 unrelated families. Mutational analysis was performed in 24 families diagnosed with LCA, 53 with early onset Retinitis Pigmentosa and 34 not-early-onset Retinitis Pigmentosa. Samples were studied with a microarray (Asperbio) followed by a family study and direct sequencing in the laboratory.

The respective frequencies of mutant alleles are: 23% (11/48) for LCA with 7 mutated families (one carried an homozygous mutation, two are compound heterozygotes, three are single heterozygotes, and one is digenic, possible triallelism), 15% (16/106) for early-onset ARRP with 13 mutated families (one carried an homozygous mutation, ten are single heterozygotes and two are digenic, possible triallelism) and 6%

(4/68) with 4 mutated families with ARRP not-early-onset (all four are single heterozygotes). CRB1 is the most mutated gene in Spanish affected families.

The combination of microarray and laboratory analysis is an optimal option for finding new disease alleles and it allows detecting cases of possible triallelism.

There is a gradient in mutation frequencies with respect to onset and severity of retinal disease, so correct classification of families is fundamental.

The relative percentage of mutations found with the microarray suggests that more LCA-associated genes remain to be discovered.

P0956. Rapid identification of female haemophilia A carriers with deletions in the F8 gene by quantitative Real-Time PCR analysis.

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Patients with haemophilia A have a bleeding disorder caused by a deficient function of the coagulation factor VIII. The identification of carriers of the disease is an essential part of genetic counselling and prenatal diagnosis. The present approach to carrier diagnosis of HA depends on the type of mutation involved in the family. Large deletions of the F8 gene account for approximately 5% of severe HA patients. Although deletions are readily detectable in males, the identification of heterozygosity in possible carriers of these families still constitutes a challenge. In order to identify a deleted allele over the background of the normal allele in these carriers, we developed a rapid real-time quantitative PCR approach by means of LightCycler technology and SYBR green I for monitoring product formation. The method was applied to families with independent deletions (one in exon 14 and the other in exons 23-24) of the F8 gene, thereby allowing a reliable determination of carrier or non-carrier status. The method is extremely versatile and can be adapted to other deletions within the F8 gene as well as to other diseases whose molecular pathology consists of deletions or duplications.

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P0957. Alpha-Thalassemia molecular determinants

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The α-thalassemias are probably the commonest monogenic condition worldwide. However, because of the restricted distribution of their more severe forms, they pose a less serious global health problem than the β-thalassemias. Nevertheless, the milder alleles which reach extremely high frequencies in some populations are cause for concern. Because many of them result in varying degrees of hypochromic anemia they are frequently mistaken for iron deficiency. Furthermore, they may have quite profound effect on the phenotypes of different varieties of β-thalassemia. Most of the deletion types (-a or -- genes) could be determined by GAP-PCR in a multiplex format. The point mutation alleles are analyzed by ARMS-PCR. In this manuscript we describe the result of molecular screening for α-thalassemia mutations in 350 suspected carrier individuals. Most of those individuals are from south of Iran. The mutation for one third of the cases could not be characterized by the routine PCR approach. Amongst those diagnosed, the most frequent allele was the mild -a^{3.7} in both heterozygous and homozygous forms with 65% and 17.5% respectively. This allele is also common in other populations in the region. The other common mild allele, i.e. the -a^{4.2}, had a much less frequency of around 2%. The point mutation allele a^{5NT del} allele was observed at 7% frequency. From the severe alleles of -a^{20.5} and -MED, each were observed at 4% frequency. Further work is underway to fully characterize the complete spectrum of α-thalassemia mutations.

P0958. The S allele of alpha-1-antitrypsin associates milder lung disease in cystic fibrosis patients under 15 years old

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The severity of the pulmonary disease in cystic fibrosis patients cannot

be predicted in patients with severe CFTR mutations partially due to the influence of genetic modifiers.

The aim of this study was to investigate whether deficient alleles of A1AT modulates the respiratory disease in cystic fibrosis patients.

Sixty one cystic fibrosis patients were initially included in the study. CFTR mutations and the S and Z alleles of the A1AT gene were genotyped. In order to minimise the role of the CFTR genotype, patients with mild mutations were excluded of the study. Data corresponding to the basal forced expiratory volume in 1s (FEV1), Crispin-Norman score and forced vital capacity (FVC) under 15 years of life were retrospectively recorded

Twenty patients carried at least one copy of the deficient variant type S. No patients bearing the Z allele were found.. FEV1 values were higher in patients bearing the S allele. FVC values and Crispin-Norman scoring showed a trend to better results within this group.

S allele is associated with better pulmonary function in cystic fibrosis patients with pancreatic insufficiency under 15y of age and FEV1 is the functional indicator showing the best correlation with the presence of the S allele.

P0959. Rapid screening of Beta-thalassaemia mutations by heteroduplex analysis with capillary array electrophoresis (HA-CAE) in the population of Castilla-Leon region (Spain)

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We present a new method to detect mutations in the Beta-globin gene based in heteroduplex analysis by capillary array electrophoresis.

In the last years more than 150 molecular defects in the human b-globin gene have been characterized. The gene maps to human chromosome 11 and consists of three exons separated by two introns. Mutations in the b-globin gene sequence can reduce the b-globin protein synthesis (β^+ thalassaemia) or even to abolish it completely (β^0 thalassaemia). These alterations in the β -globin chains synthesis are an important health problem, specially among the Mediterranean population.

We have analysed 47 DNA samples of patients with thalassemic trait (heterozygotes) : microcytosis and anomalous HbA₂, and we have characterized 34 mutations (72,34%) analyzing only two exons of the B-globin gene, showing that the most frequent mutation in this population is CD 39 C-T substitution. These results are in agreement with those obtained by the DGGE method (Denaturant Gradient Gel Electrophoresis), but the mutation detection rate of this last method was only of the 70%. Furthermore, the possibility of use a multiplex PCR for the two exons and the reproducibility of electropherograms for each mutation pattern is an advantage for the mutation screening in the beta-globin gene in our population.

P0960. Analysis of HFE1 gene mutations in a group of steel makers and in a cohort of CF patients from Ukraine

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Hereditary haemochromatosis is the prototype disorder of iron overload due to misregulated iron homeostasis in humans. That may result in severe systemic disease, such as cirrhosis, liver disease, pancreatic insufficiency, cardiomyopathy, diabetes mellitus, etc.

In our study we compared the prevalence of the genotype C282Y/+ in a group of 49 patients with pancreatic insufficiency and diabetes mellitus and a control group (n=97). The prevalence in the patients (30.9%) was significantly increased (p=0.032). Interestingly, all the patients worked in conditions of exogenous iron overload (steel makers, welders).

In order to investigate the possible role of HFE1 gene mutations in modulation of the severity of gastrointestinal insufficiency in patients with cystic fibrosis the C282Y and H63D mutations were analyzed in a cohort of 35 CF patients with severe gastrointestinal insufficiency and/or liver disease. The frequency of the C282Y mutation was the same in the control and CF groups. The frequency of H63D in the control and CF groups was 17% and 30% respectively. This difference is statistically significant (p=0.01), and the prevalence of the genotype H63D/H63D was significantly elevated in the CF group (p=0.05). Our data may show evidence of a possible role of HFE1 gene mutations in the pathogenesis of CF.

P0961. Epigenetic regulations of the FMR1 gene: the role of the histone code.

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The fragile X syndrome is caused by a >200 CGG repeat expansion within the FMR1 gene promoter, with consequent DNA hypermethylation and inactivation of its expression. To clarify the mechanisms that suppress the activity of the mutant gene, we investigated the acetylation and methylation status of histones in three regions of the FMR1 gene (promoter, exon 1 and exon 16) in fragile X cell lines, using chromatin immunoprecipitation (ChIP) coupled with real-time PCR. Basal levels of histone acetylation and H3-K4 methylation were much higher in transcriptionally active controls than in fragile X cells. Treatment of fragile X cells with 5-aza-2-deoxycytidine induced a decrease of H3-K9 methylation, an increase of H3 and H4 acetylation and an increase of H3-K4 methylation. On the other hand, treatment with acetyl-L-carnitine, which does not reactivate the FMR1 gene, resulted in increased histone acetylation, in spite of persisting DNA methylation and low H3-K4 methylation. Finally, the analysis of a cell line (5106), derived from a rare individual of normal intelligence with an unmethylated full mutation, revealed that this cell line expresses normal mRNA levels and reduced levels of FMRP protein (-30%). The epigenotype of line 5106 is surprising, with H3-K4 methylation comparable to controls, but deacetylated histones and methylated H3-K9, like fragile X cells, in both the promoter and exon 1. Our experiments indicate that H3-K4 methylation and DNA demethylation are the main epigenetic switches activating the expression of the FMR1 gene, with histone acetylation and H3-K9 methylation playing an ancillary role.

P0962. Molecular and methylation analysis of the FMR1 gene promotor and repeat region in twelve fragile X Spanish families

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Fragile X syndrome (FRAXA) is the most common cause known of inherited mental retardation. Although no treatment is yet available the diagnosis of this syndrome in a child is essential in order to provide the family with genetic counselling. Because of this, molecular screening programs in mentally retarded individuals have been performed in several regions of Spain. Its variable phenotypic expression makes the clinical suspicion and genetic diagnostics difficult, especially for premutation and intermediate alleles. New techniques in DNA analysis like methylation-sensitive PCR (MSP), novel approaches in DNA and bisulphite sequencing allow both affected individuals and carriers to be tested in a more precise way from a simple blood sample, and assist research about the molecular mechanisms involved. We have recently applied these techniques in 12 families with fragile X syndrome in order to improve fragile X diagnostics and do a retrospective study of the transmission of intermediate, premutation and full mutation alleles, to reach a better understanding of trinucleotide CGG repeat expansion. By means of repeat region sequencing we have analyzed the absence of intervening AGG interruptions associated with instability of intermediate and premutation alleles. Methylation-sensitive PCR and bisulphite sequencing allow us to study the methylation status of the FMR1 gene promoter and CpG island repeat in these patients to attempt to understand the epigenetic mechanisms implicated in FMR1 gene regulation in order to acquire a better understanding of the molecular basis of FRAXA.

P0963. Hereditary hemochromatosis mutation S65C prevalence in general population of Latvia and patients with iron overload

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Background. The hereditary hemochromatosis (HH) is an inborn disorder characterized by iron overload of parenchymal organs. The disease is caused by mutations of gene HFE. The mutation S65C is found on 7.8% of patients' chromosomes that lack the two most

common mutations C282Y and H63D, and is associated with a milder form of the disease. The population frequency of the mutation S65C varies between populations. The frequency of HH mutations C282Y and H63D in healthy Latvian population has been reported earlier and is 3.5% and 12.1% respectively. **Objectives.** To establish the mutation S65C frequency in population of Latvia, and to analyze DNA of patients with iron overload for mutations C282Y, H63D and S65C. **Materials and methods.** The DNA analysis was performed in a group of 155 healthy residents of Latvia and in 48 patients with iron overload. The DNA was extracted from whole blood, PCR, restriction enzyme digestion and polyacrylamide gel electrophoresis was used for mutation analysis. **Results.** Of 310 chromosomes of general population examined, 3 carried the mutation S65C. The resulting mutation frequency is 0.0097, with a heterozygous carrier frequency of 1.9% or \approx 1:50. Of 48 patients with iron overload, 5 were confirmed to have hereditary hemochromatosis, the detected genotypes were: C282Y/C282Y - 2 patients, C282Y/H63D - 2 patients, H63D/S65C - 1 patient. 2 of the rest were heterozygous for mutation C282Y, 11 - for mutation H63D, and 1 - for mutation S65C. The resulting frequency of mutation S65C among patients with iron overload is 0.021.

P0964. Expression studies of *Sall4* and its interaction with *Sall1*

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Sall4 belongs to the *spalt* (*sal*) family of proteins, which are characterized by their unique multiple double zinc fingers motifs. *Sall* proteins act as transcriptional repressor factors and have been implicated in development and disease. Four members of *Sall* family have been identified in mouse and human. Mutations in *SALL4* are associated with Okihiro syndrome, an autosomal dominant disorder characterised by forelimb defects and Duane anomaly. *SALL1* mutations cause Townes-Brocks syndrome which has an overlapping phenotype with Okihiro syndrome. Our investigation is focused on the characterization of *Sall4* expression in mouse and its interaction with *Sall1*. In the previous studies strong expression of *Sall4* was found in the developing neural tube and limbs. In present study we report new alternative splicing variants of *Sall4*, transcripts which we named *Sall4* major and *Sall4* minor. *Sall4* minor variant contains a unique exon instead of exon 1 of *Sall4* major, which we named exon 1a. Exon 1a encodes for a translation initiation site which differ from *Sall4* major only in amino acids (1-40). Expression during embryonic development for both transcripts was detectable as early as E7.5 and gradually decreases until E17.5. Real-time RT-PCR analysis revealed lower expression level of *Sall4* minor variant in compare with *Sall4* major. To study the interaction of *Sall4* with *Sall1* we use biochemical approach. Epitope tagged *Sall4*, *Sall1* and truncated *Sall1* (dominant negative fragment) were transfected into NIH 3T3 cells and interaction were studied by co-immunoprecipitation and co-localization experiments. The results will be presented in the poster.

P0965. Molecular analysis of Czech and Slovak patients with Methylmalonic aciduria

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Methylmalonic aciduria (MMA) is an autosomal recessive disorder caused by a functional defect in the nuclear encoded mitochondrial enzyme methylmalonyl-CoA mutase (MCM, EC 5.4.99.2, gene *MUT*) which catalyzes the isomerization of L-methylmalonyl-CoA to succinyl-CoA. MMA can also result from the defects in the synthesis of MCM cofactor adenosylcobalamin: *cblA* is caused by the defect in the mitochondrial *Cbl* transport (gene *MMAA*) and *cblB* is caused by the defect in the *cob(I)alamin* adenosyltransferase (EC 2.5.1.17, gene *MMAB*).

We have examined eight patients with MMA. Enzyme assay revealed five patients with mut MMA and three with defect of *cblA/B*. Subsequently we detected 16 mutated alleles. On the basis of molecular studies we were able to distinguish between the *cblA* (one patient) and *cblB* (two patients).

Four novel mutations were found in the *MUT* gene - two novel missense mutations (c.1881T>A and c.1105C>T), one nonsense (c.2179C>T)

and one splicing mutation. The splicing mutation is caused by unknown intronic mutation. It leads to two mRNAs of different length caused by deletion of exon 4 in the first transcript and deletion of exon 4 and part of the exon 3 in the second transcript. One novel indel mutation (c.558_559delGGinsC) was found in the *MMAB* gene and one novel insertion (c.551insG) was found in the *MMAA* gene.

The results of molecular analyses have been already used in the prenatal diagnoses in two affected families.

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P0966. Molecular diagnostics of pathogenic fungi in patients treated with hormonal replacement therapy.

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Fungal infections of oral cavity are very common in general population (30-40% occurrence) especially in patients with dental wears. Medical treatment, especially immunosuppression, could be additional risk factor of fungal infection. Hormonal replacement therapy could result in nonspecific immunomodulation probably caused by leukocyte activation dependent on estrogen receptor polymorphism. Molecular methods of identification of pathogenic fungi are universally applicable in contrast to limitations of morphological characteristics. Our aim of study was recognition of pathogenic fungi by molecular analyses and correlation between the presence of fungi and estrogen receptor polymorphism known as a Pvull and XbaI polymorphisms in control group and group of patients treated with hormonal replacement therapy. DNA was isolated from vaginal and oral swabs. A panel of 100 DNA derived from normal individuals was used to establish allele frequencies for control population. Universal primers, targeting the conserved regions of ITS1 and ITS2 respectively, were used for amplification. DNA pattern from known fungal strains was established for comparison with clinical samples. PCR products were analyzed with real-time PCR using Lightcycler System. Results show the presence of *Candida albicans* with or without *Candida Glabrata* in almost all clinical samples. In several cases analyses revealed infection with more than two fungal species. The allele frequencies for the Pvull and XbaI RFLPs in our control population were found as follows: P allele 0.48; p allele 0.52; X allele 0.37; x allele 0.63. In group of patients frequencies were similar, but frequency of infection with more than one fungal species was higher.

P0967. Analysis of gene expression in *Pax3* null mouse embryos using Affymetrix microarrays

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Pax3 is a paired box containing transcription factor expressed in the region of the dorsal neural tube that gives rise to migrating neural crest populations. Mutations in *Pax3* result in neural crest defects in mouse and man. Heterozygous mutations in human *PAX3* give rise to Waardenburg syndrome which comprises of deafness and pigmentary defects of the hair, skin and iris. *Pax3* null mouse embryos exhibit conotruncal heart defects and abnormalities of the thymus, thyroid and parathyroids. They also suffer from defects of neural tube closure such as spina bifida and exencephaly and have abnormal development of their dorsal root ganglia, sympathetic ganglia and somitic tissues. To understand the genetic aetiology of the defects seen in the *Pax3* null mouse, RNA was extracted from the branchial arch region of 3 e10.5 embryos and hybridised to Affymetrix microarray chips (wild type n=3, *Pax3* null n=3). The expression profiles of the chips were analysed statistically to arrive at a list of significantly changed genes. Several genes involved in outflow tract formation, neural tube closure, neurogenesis and somite development were found to be significantly up or downregulated in *Pax3* null mouse embryos. Some of these genes changes were verified by RTQPCR. This study contributes to the understanding of defects in mammalian neural crest migration and provides candidate genes for potential *Pax3* targets.

P0968. A new splicing isoform of LOX-1 is protective against acute myocardial infarction

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Lectin like oxidized LDL receptor-1 (LOX-1) is a scavenger receptor formerly identified as a membrane receptor for oxidized LDL (OxLDL). LOX-1 activation is associated with endothelial and monocyte/macrophage apoptosis and adhesion of monocytes to activated endothelial cells. Independent association genetic studies have demonstrated the implication of LOX-1 gene variants in myocardial infarction susceptibility (MI). Since single nucleotide polymorphisms (SNPs) linked to MI are located in intronic sequences of the gene, it remains unclear as to how they determine their biological effects. We show that intronic SNPs associated with MI regulate the expression of a new functional splicing isoform of the LOX-1 gene (LOXIN) lacking exon5. Using quantitative Real-Time PCR and minigene approach, we demonstrate that subjects, carrying the disease susceptible haplotype at LOX-1 gene, show a decreased expression of LOXIN mRNA and an increase in the expression of LOX-1. The hypothesized protective effect of LOXIN was investigated by analysis of the macrophage apoptosis induced by OxLDL. Flow cytometric analysis showed a 24% reduction in apoptotic cells number in subjects carrying the "no risk haplotype". These data confirm that the increase of LOXIN mRNA protects cells from apoptosis supporting the protective role of LOXIN overexpression against MI. To investigate the cellular localization of LOX-1 and LOXIN, we tagged both cDNA sequence with that of the green fluorescent protein (GFP) showing an impaired intracellular traffic to the plasma membrane of the LOXIN-GFP fusion protein. Our findings provide evidence for a direct link between the LOX-1 expression and the pathogenesis of MI.

P0969. 10 years of DNA diagnostic services in Estonia

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Molecular Diagnostic Center was established in 1996 for DNA testing and newborn screening for phenylketonuria and hypothyroidism for whole Estonia. Since 2001 it belongs to the United Laboratories of Tartu University Clinics. Introducing the DNA diagnostics was started from cystic fibrosis. Duchenne/Becker muscular dystrophy and Fragile X syndrome were next obvious choices. By 2005, the number of tested disorders has increased to 28 (www.dnatest.med.ee). The most recent DNA tests are Stargardt disease, Usher syndrome, retinitis pigmentosa, CF, deafness, Leber congenital amaurosis where several hundred mutations are analysed using APEX (arrayed primer extension) microarrays (collaboration with Asper Biotech).

The Estonian population is 1,35 million with 14 053 newborn in 2004. More than 3000 patients have been tested up to now. The most frequent test is one for cystic fibrosis and/or CBAVD. Two most common mutations in CF gene are F508del and 394delTT accounting for 54% and 14 % of all CF chromosomes. Another big group of patients are tested for Fragile X syndrome. Altogether 13 patients were found. Interestingly, from four spinocerebellar ataxias 11 patients were diagnosed as SCA2, and only one as SCA1. The largest number of tests performed last year was factor V Leiden and prothrombin gene G20210A mutations as risk factors for thrombophilia, both introduced in 2004. 22 patients out of 95 were factor V Leiden heterozygous, 6 out of 75 were prothrombin G20210A heterozygous. We will give comprehensive data about the DNA testing in Estonia for all tests and their dynamics over the 10 years.

P0970. Altered patterns of *Edar* and *Xedar* expression in *Mus musculus* and *Rattus norvegicus*

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Anhidrotic ectodermal dysplasia results from a defect in the differentiation of skin appendages during embryonic development. Affected individuals may exhibit symptoms of anodontia or oligodontia, hyperthermia and hypotrichosis (sparse hair). Several animal models have been described that exhibit symptoms similar to those seen in affected patients. Results support the hypothesis that the *Edar* and *Xedar* genes encode proteins involved in pathways transducing signals from ectoderm to mesenchyme, which initiate formation of skin appendages. Recently we have developed comparative analysis of mouse and rat models of *Edar* and *Xedar* gene expression during embryonic development. Formalin-fixed, paraffin-embedded specimens of *Mus musculus* (8-16 day embryos) and *Rattus norvegicus* (10-18 day embryos) were obtained from Novagen. The tissues were chosen to show different development of hairs or scales in the same parts of the body like the tail. Total RNA was isolated from epidermal cells microdissected from embryonic tails according to standard laboratory procedures. RNA was used as a template for real-time RT-PCR with a Lightcycler System. Results after normalization with β -actin expression (detected in all samples) were placed in an Excel spreadsheet. Analysis revealed almost the same expression profile of *Edar* in both mouse and rat epidermis. Profiles of *Xedar* gene expression were different: in the mouse epidermis the level of expression is almost constant during all analyzed days of embryogenesis, in the rat the level of expression increases significantly during embryogenesis. Our results suggest strong correlations between timing of expression and development of skin appendages.

P0971. Mosaic dup(17)p11.2 CMT1A: not as rare as previously thought?

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CMT1 is a genetically heterogeneous peripheral neuropathy, characterized by muscle weakness and atrophy and sensory loss. CMT1A represents about 70% of cases and is commonly caused by a duplication of a 1.5 Mb region including PMP22, generated by unequal crossing-over. Cases of mosaic duplications have been very rarely described and it is suggested but currently unproven that they may arise as mitotic reversion events (Liehr et al. 1996).

We have selected interphase FISH of cultured lymphocytes followed by analysis of >100 nuclei as the diagnostic method of choice, to allow detection of such mosaics. The duplication was identified in 9/51 individuals (18%), 4 of whom exhibited somatic mosaicism. The patients were aged 72, 51, 22 and 19 years of age, with 47%, 52%, 33% (two independent samples) and 81% of duplicated nuclei respectively. Patient 1 was mildly affected and had a more severely affected son whose test revealed no mosaicism (100% duplicated). Patient 2 had a mother with "polyneuritis" who could not be tested. Patient 3 has a mother with a clinical diagnosis of CMT but who has not been genetically tested. Patient 4 has a negative family history. Such a high incidence of mosaicism is surprising. It may be due to chance, to a selection bias (testing of less-affected patients), or to our analytical approach. Liehr et al. found that rapidly-dividing cells had a lower frequency of duplications; our use of cell culture and analysis of a large number of nuclei may reveal mosaicism that would otherwise be missed.

P0972. Molecular analysis of CX26, D(GJB6-D13S1830) and A1555G mitochondrial point mutation in Italian deaf population and occasional findings of CX26 mutations in partners of deaf or carrier subjects.

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More than 100 different mutations of Connexin 26 are described in non-syndromic recessive deafness but one is particularly common in our population, the 35delG that accounts for up to 60% of mutated GJB2 alleles. We analysed 448 NSRD patients and identified mutations in 288/896 chromosomes; 32,1% (191/896) showed 35delG, while the remainder showed many different mutations: -3170G>A, 31del14, G12V, 35insG, W24X, M34T, V37I, A40G, E47X, W77R, V84M, L90P, V95M, H100L, 167delT, 290insA, 310del14, delE120, W133X, E147K, C174R, D179N, R184P and R184W. 35delG was present in about 66% (191/288) of all Cx26 mutations identified. We investigated also the del(GJB6-D13S1830), a 342 kb deletion in 13q12, including exon 1 of GJB6 coding Connexin 30. We have found two compound heterozygotes both carrying del(GJB6-D13S1830) in association with the 35delG and the 167delT GJB2 mutations, respectively. Our results show that GJB2 mutations account for less than 50% of recessive non syndromic hearing loss cases and confirm that the 35delG mutation is the most frequent one, but many other mutations are also present such as M34T, E47X, L90P and delE120. Moreover 12 affected subjects were compound heterozygous for recessive GJB2 allele not including 35delG and 4 were carrying the D179N dominant mutation, indicating that the complete sequence of the gene is needed for an appropriate molecular diagnosis. We found 3 affected subjects carrying the A1555G and the subsequently family analysis performed in each case has led to the pre-symptomatic identification of this mutation in 5 relatives.

P0973. Mutation analysis of LEMD3 in osteopoikilosis, Buschke-Ollendorff syndrome and melorheostosis patients

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Osteopoikilosis is an autosomal dominant skeletal dysplasia characterized by multiple hyperostotic areas in different parts of the skeleton. Osteopoikilosis can occur either as an isolated anomaly or in association with other abnormalities of skin and bone. Buschke-Ollendorff syndrome (BOS) refers to the association of osteopoikilosis with connective tissue nevi. Osteopoikilosis can also be associated with melorheostosis. The latter disorder is characterized by a "flowing" hyperostosis of the cortex of tubular bones. After a genome wide linkage analysis in three families, we were able to identify *LEMD3* as the causal gene for this group of disorders. In each family, affected individuals had osteopoikilosis with or without manifestations of melorheostosis or BOS. *LEMD3* codes for an integral protein of the inner nuclear membrane. We showed that *LEMD3* has the capability of antagonizing both BMP and TGF β signaling in human fibroblasts.

We present the results of mutation analysis of the *LEMD3* gene in a series of 13 patients. In 9 patients manifestations of BOS were present, 3 patients had melorheostosis and 1 patient had both signs of osteopoikilosis and melorheostosis. In 11 patients, a *LEMD3* mutation was identified. All mutations were predicted to result in haploinsufficiency of the protein. In 3 patients (2 patients with melorheostosis and 1 patient with BOS) no mutation was found in the coding sequence of *LEMD3*. We are currently sequencing in these patients the non-coding parts of the *LEMD3* gene. We are also investigating the possibility of a somatic *LEMD3* mutation in osteoblasts from one patient with melorheostosis.

P0974. Prokaryotic and Eukaryotic Expression Studies of MCPH1

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Primary microcephaly can be due to gene defects at any one of six recently reported microcephalin loci, MCPH1 to MCPH6.. Corresponding patients show reduction of their cerebral cortex volume and mental retardation. In addition, defects of the MCPH1 gene, located on chromosome 8p22, result in premature chromosome condensation in the G2 phase (PCC syndrome). The MCPH1 gene encodes a polypeptide encompassing 835 aa. The MCPH1 protein contains one N-terminal and two C-terminal BRCT domains linking its function to DNA checkpoint control and/or DNA repair. We cloned two ORFs of MCPH1 cDNA, 1-2508 and 286-2508, into the bacterial vector, pIVEX1.3, for prokaryotic protein expression in the cell-free E.coli lysate system. Expression of the large ORF gave rise to two translation products of 93 and 80.5 kDa. The latter variant corresponded to the translation product of the shorter MCPH1 ORF. This finding suggests that the internal ATG at 286 may be used as a translation initiation site, although the shorter product was present when the large MCPH1 ORF was cloned into the pIVEX vectors for cell-free eukaryotic expression in wheat germ extract and rabbit reticulocyte lysate. We also cloned the large and small variants of MCPH1 as bi-cistronic constructs with the neomycin resistance gene into a retroviral vector. Studies are underway to show if one or both species are able to complement MCPH1 +/- human cell lines. With tools for expression at hand interactions and cell cycle effects, may be studied in MCPH1 proficient vs. deficient states of the same cell line.

P0975. Molecular and demographic analysis of the cystic fibrosis population in Estonia.

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Our aim was to analyze the molecular and demographic data of the CF population in Estonia in terms of age, gender, age at diagnosis, ethnicity, survival and mortality rates during last decades and to investigate the clinical manifestation of CF patients in relation to their genotype. We aimed to include all CF patients (n=80) in Estonia born between 1974-2004, but clinical and molecular data was available for 45 patients from 40 families.

The incidence of CF in Estonia by archival data is 1:7750 and according to the Hardy-Weinberg calculations 1:7450 live-births. Several common CFTR mutations were detected: F508del (54% of all chromosomes), 394delTT (14%), 3659delC (2%), 1716G>A (2%). Additionally, 14 mutations were found only in one allele. Altogether, 87.5% of all mutated CF chromosomes were identified. New methods like arrayed primer extension (APEX) allowing parallel detection of numerous mutations should be introduced to reach mutation detection rate close to 100%.

In 2003 the mean age of the living patients known by us was 12 years 3 months (95% CI 113-182 months) and 29% of our patients were over 18 years old. The mean age of establishing CF diagnosis was 2 years 3 months (95% CI 18.0-36.1 months). The mortality rate of CF patients in Estonia is decreasing, being 12.2% during the period 1983-1987 and dropping to 0.7% in the period 1997-2002. Although, there is a tendency of ageing of our CF population, more effort should be made to diagnose CF in earlier age and to give better treatment in specialized centrum.

P0976. Functional identification of a classical nuclear export signal in spastin (SPG4) and generation of constructs targeting over-expressed protein to the nucleus

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Mutations in SPG4, the gene encoding spastin, are responsible for

the majority of cases of hereditary spastic paraparesis (HSP). Native spastin is detected mainly in the nucleus, and two basic amino acid stretches from within the protein have recently been shown by us to act as nuclear localisation signals (NLSs); over-expressed spastin, however, is restricted to the cytoplasm. Actual protein function in either compartment as well as the mechanism(s) underlying pathogenicity of mutations remain to be elucidated. To investigate the role of nuclear spastin, we set out to direct over-expressed protein to the nucleus by adding yet another strong NLS; we observed that full length constructs still fail to localise to the nucleus. We identified an N-terminal region conferring this effect and subsequently showed that it contains a functional, exportin 1-dependent nuclear export signal (NES). Based on these findings, we applied site-directed mutagenesis of a single critical leucine residue from within the NES to generate constructs finally directing over-expressed protein to the nucleus. Our study adds to understanding the intra-cellular distribution of spastin and prepares the ground for further studies into its physiological role. It may therefore help to, eventually, fully link SPG4 mutations to HSP pathology.

P0977. Point mutation in the STS gene in a severe affected patient with X-linked ichthyosis

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X-linked ichthyosis (XLI), an inherited disorder characterized by scaly skin, is due to steroid sulfatase (STS; EC3.1.6.2) deficiency and occurs 1 in 2000 or 6000 males. Onset is at birth or during the first months of life with the presence of dark, regular, adherent scales of skin. XLI diagnosis can be established through the STS assay. STS gene is located on Xp22.3. Most XLI patients harbor complete deletion of the entire STS locus and flanking markers. In some cases, abnormal paring of the low-copy-number repeats G1.3 and CRI-S232, on either side of the STS gene, seems to be responsible of these interstitial deletions. Only a few patients have been reported with partial deletions or point mutations in the STS gene. In the present study we analyzed a severe affected XLI patient with a missense mutation in exon 8 that seems to be a critical region in the presence of point mutations in XLI.

P0978. MDR3 gene analysis in children with Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3) phenotype

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Human MDR3 gene encodes the class III multidrug resistance P-glycoprotein that mediates the translocation of phospholipids across the canalicular membrane of the hepatocyte, a process that is of crucial importance in protecting cholangiocyte membranes from high concentrations of detergent bile acids. MDR3 mutations are cause of Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3), characterized by early onset of cholestasis that progresses to cirrhosis and liver failure before adulthood, with high serum gamma-glutamyltransferase (gGT) activity; the liver histology shows ductular proliferation and inflammatory infiltrate in the early stage despite patency of intra and extrahepatic bile ducts.

We analysed 33 children with a PFIC3 phenotype by sequencing the entire coding sequence of MDR3 gene and we identify 10 pathogenetic alleles and twelve mutations in six children. Eleven are single different mutation and all are unpublished. In particular, in two patients only one mutation was identified, and in other two children we identify three mutations, two of which on the same allele.

Four mutations imply the synthesis of a truncated protein. In particular we identify 9 missense mutations (Arg159X, Ala250Pro, Glu558Lys, Met 630Val, Leu701Pro, Thr715Ile, Gly723Glu, Glu888X e Ala1186Thr) and 2 insertions (1167insAA and 2200insG). Exon 17 could be identified as a hot-spot since it holds four of the eleven mutation identified (36.4%).

None of the eleven mutation is present in 100 alleles of the control population. The low detection rate of MDR3 mutation in our patients (about 18%) could be due both to genetic and clinical heterogeneity.

P0979. Molecular and biochemical basis of alcohol-induced oxidative stress in rat's lungs

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In forensic practice intoxications with alcohols are very common. Recently authors evaluated rat model of intoxication to establish biochemical basis of alcohol poisoning. Our aim was investigation of the influence of methanol, ethanol, and ethylene glycol on oxidative stress in rat's lungs and evaluation of the tissue expression of catalase and superoxide dismutase. Male Lewis rats had been administered 1M methyl alcohol or 1M ethyl alcohol or 0,25M ethylene glycol for 4, 8 and 12 weeks. In rat's lungs we measured the concentrations of hydrogen peroxide, lipid peroxidation products (TBAR) and antioxidant system components (SH-groups). Results are represented in count per milligram of protein (Lowry's method). Isolation of RNA from rat's lungs has been made according to the laboratory procedures. RNA was used as template for real-time RT-PCR. Results after normalization with β -actin expression have been analyzed with Excel spreadsheet. Our results suggest that ethanol, methanol and ethylene glycol generate oxidative stress in rat organs and may influence on antioxidant enzyme activity causing lipid peroxidation and protein damage, and increase hydrogen peroxide production. These parameters were changing especially in erythrocytes, liver, kidney, brain, lung. The alcohol generating the oxidative stress in all examined organs most significantly, was ethylene glycol. Examination of catalase and superoxide dismutase expression profiles revealed significant differences in analyzed groups of rats due to intoxication with different alcohols.

Results										
	Methanol			Ethyl alcohol			Ethylene glycol			
	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	4 weeks	8 weeks	12 weeks	
SH	89,41663	57,00633	51,31375	167,4597	48,36255	50,31624	44,91551	20,68021	32,74381	
statistic	p<0,001	ns	ns	p<0,01	ns	ns	ns	p<0,01	ns	
TBAr	4,654119	3,507508	2,529495	4,006854	3,796295	2,551526	4,611628	9,269838	7,61818	
statistic	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	p<0,001	
Hydrogen peroxide	88,83909	142,9098	129,3559	145,955	127,3569	75,21629	60,97764	86,50598	761,8356	
statistic	ns	ns	p<0,05	p<0,05	p<0,05	ns	ns	ns	p<0,01	

P0980. ABCA1 gene polymorphism and lipid profile in a Scottish population

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The ATP Binding Cassette Transporter (ABCA1) is a protein involved in lipid metabolism. Common polymorphisms in this gene could affect High Density Lipoprotein Cholesterol (HDL-C), triglyceride and Apolipoprotein A1 (apoA1) levels and thus the risk of atherosclerotic diseases. The purpose of this study was to assess the effects of different ABCA1 genotypes on lipid profile. A cohort of 181 healthy Scottish individuals with complete lipid profile data (adjusted for age, sex, BMI and smoking, drinking habits) was recruited. We studied four common polymorphisms in ABCA1 gene at different positions, G158A (Leu/Leu), G219A (Arg/Lys), G316A (Gly/Gly) and A1587G (Lys/Arg). Genotyping assays were based on Dynamic Allele Specific Hybridisation (DASH).

Results:

No differences were found in VLDL/TG, VLDL/Cholesterol, LDL, HDL2 and HDL3 concentrations in females or males between different genotypes. However, in females with different A1587G genotypes IDL was higher in "AA" genotype (table1). It was also higher in females with G219A "GG" genotype (table2). Males with G316A "GA" genotype also had a higher level of HDL (Mean HDL: "GG", 53.27; "GA", 63; p=0.032). In conclusion, only A1587G "AA" and G219A "GG" genotypes may influence IDL in females. G219A "GG" genotype might have an effect on total HDL in males only.

Table-1: IDL in different A1587G genotypes (females); P=0.016

Genotype	Number	IDL(mg/dL)	Log (\pm SD)Mean
11 (GG)	43	12.60	1.10 (\pm 0.22)
12 (GA)	36	14.81	1.18 (\pm 0.18)
22 (AA)	3	23.33	1.44 (\pm 0.31)

Table-2: IDL in different G219A genotypes (females); P=0.013

Genotype	Number	HDL2(mg/dL)	Log (\pm SD)Mean
11 (GG)	27	17.70	1.27 (\pm 0.16)
12 (GA)	21	12.05	1.07 (\pm 0.23)
22 (AA)	4	13.25	1.11 (\pm 0.23)

P0981. CRYGD gene mutation in a new family with congenital hereditary aculeiform cataract

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Autosomal dominant congenital aculeiform cataract is one of the rarest types of inherited cataract and is characterized by fiberglass-like or needle-like crystals projecting in different directions, through or close to the axial region of the lens. Previously, crystallin Gamma D gene (CRYGD) mutations were demonstrated in 3 families (two from Switzerland and the other one from Macedonia) with aculeiform cataract; interestingly, the same Arg-58 to Hys mutation was identified in these three unrelated pedigrees. Here, we report the results of CRYGD molecular analyses in a large Mexican family with aculeiform cataract. The propositus is a female aged 23 years who asked for medical advice because of "low visual acuity" from childhood; familial history disclosed that several relatives were previously diagnosed as having congenital cataracts; on eye biomicroscopy, a white central opacity resembling glass, originating from the fetal nucleus and radially projecting needle-like crystals, was observed in both lenses. Fourteen relatives were clinically examined and 7 of them were identified as suffering from the same type of bilateral cataract.

Molecular analyses on genomic DNA from the propositus and from 5 affected relatives demonstrated a G to A heterozygous missense mutation in position 411 in exon 2 of CRYGD, which originates a Arg-58 to Hys substitution in the protein. The Arg-58 to Hys substitution identified in this family is identical to that previously demonstrated in three unrelated families with aculeiform cataract. These data suggests that there is a strict genotype-phenotype correlation in this type of congenital hereditary cataract.

P0982. Evidence for a New Locus for Bardet Biedl Syndrome

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Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterized by obesity, retinopathy, polydactyly, renal and cardiac malformations, learning disabilities, and hypogenitalism. Eight BBS loci have been mapped, and eight genes have been identified. We investigate linkage and haplotype analysis in two consanguineous Tunisian families. METHODS. Haplotype and linkage analysis of two consanguineous families diagnosed BBS was scored with more than 24 polymorphic markers neighboring and surrounding the eight known BBS genes. Two-point lod scores were calculated. DNA from affected individuals from both families was amplified with primers specific for BBS1, BBS2, BBS4, BBS6, BBS7 and BBS8 genes and then sequenced by direct automated sequencing. RESULTS. The combined haplotype analysis and linkage analysis in two Tunisian consanguineous families with BBS have excluded any linkage to any of the eight known BBS loci. Sequence analysis of BBS1, BBS2, BBS4, BBS6, BBS7 and BBS8 genes revealed no sequence alterations in their coding sequence. CONCLUSIONS. These results describe evidence for at least one new locus for BBS confirming the extreme genetic heterogeneity for this disease. The absence of alterations in six of the eight known BBS genes demonstrates that a new BBS locus is not involved in triallelic inheritance with these known genes.

P0983. Technical and ethical considerations in the use of MLPA technology.

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MLPA technology is a very valuable addition for molecular diagnostics however we have encountered several interesting problems.

Single exon deletions. We found an apparent deletion of one copy of exon 25 of the BRCA1 gene, but sequence analysis demonstrated that this patient was heterozygous for a mutation (R3128X) in that exon. The nonsense mutation is present in one of the MLPA primer binding sites and caused a false-positive result on MLPA. Any similar base-pair substitution in a binding site could cause an apparent false-positive result so apparent single-exon deletions should be verified by an alternate method.

Incidental findings. MLPA kits also contain control fragments or exons from other genes. We screened a patient with the MLPA kit for Rett syndrome and found a 50% reduction of a control peak. This control amplicon is from the proteolipid protein (PLP) gene that is normally involved in Pelizaeus-Merzbacher disease and spastic paraparesis 2. Although this has been useful, we note that there might have been an issue surrounding lack of informed consent. We have already encountered two similar instances: a deletion of the Fanconi D control probe in a patient with a VHL deletion indicating that the deletion was fairly large and involved a second gene that might have significance in tumour formation and a deletion of the CHL1 control probe but not any VHL exon in a patient with a cytogenetically-detected 3p deletion. Remembering these, or similar, difficulties will allow us to make better use of this excellent technique.

P0984. Familial hypercholesterolemia in St.-Petersburg: the known and novel mutations found in the low density lipoprotein receptor gene in Russia

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Familial hypercholesterolemia (FH) is one of the most common monogenic human diseases. FH is inherited as an autosomal-dominant trait with the prevalence of the heterozygous form in most populations 1:500. The main purpose of current research is to create the mutation map of the LDL receptor gene in St.-Petersburg FH patients. As a result, 32 sequence variants besides common polymorphisms were identified. Among 32 mutations, 17 were new and 27 were disease-causing mutations. All these mutations alter the reading frame of the receptor mRNA, affect splicing of mRNA or lead to changes in the amino acid sequence of the mature receptor. Rapid test methods were developed for most of the mutations. We analyzed the inheritance of the mutations in probands' families by rapid test methods. Totalizing our data, only 27 out of 66 relatives of probands were found to carry the mutations. Our results allowed to conclude that predominating mutations are absent in FH patients in St.-Petersburg. In the current study only five mutations (C139G, c.313+1G>A, c.651-653del3, c.652-654 delGGT è C308Y) were found to be recurrent, i.e. were found in two unrelated families each. Other 22 mutations were found in unique family each. Many of mutations found in St.-Petersburg were previously identified in other ethnic groups, for example in Denmark, Finland, Norway and Sweden. Most probably these mutations share the common ancestor in all Baltic countries. Current research was supported by Russian Fund for Basic Research (05-04-48235), Program "Leading Russian Scientific Schools" and by the President of Russian Federation (MK-899.2003.04).

P0985. Maternal MTHFR genotype and incidence of Neural Tube Defects (NTD) and Down syndrome

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Down syndrome (DS) and Neural tube defects (NTD) are common malformations among newborns. The C677T polymorphism in the

MTHFR gene combined with reduced dietary intake of folates has been associated with increased risk of having a pregnancy affected by NTD. Other studies suggested that abnormal folate metabolism and the C677T mutation may be maternal risk factors for DS. However, it was reported that the frequency of this polymorphism differs between different ethnic populations. In our study the frequency of C677T substitution was evaluated in DNA samples from 38 women with NTD affected pregnancy, 26 women with DS affected pregnancy and control group of 96 Bulgarian women without NTD or DS pregnancy. Differences in allele and genotype frequencies among investigated groups of NTD mothers and controls were found. The CC genotype was observed in 40% of NTD mothers, 42% of DS mothers and in 37% of control women; C/T genotype - in 50%, 42% and 45% respectively; and T/T genotype - in 10%, 16% and 18%, respectively. We suggest that NTD incidence is affected by a multifunctional interaction, including maternal folate metabolism, MTHFR genotype, and other loci. A study of the frequency of a second MTHFR polymorphism - A1298C is now in progress.

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P0986. Contribution of GJB6 (connexin 30) mutations to nonsyndromic deafness in the Portuguese population

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Mutations at the GJB2 gene, encoding connexin 26, account for up to 50% of the nonsyndromic recessive deafness cases. Nevertheless, many heterozygotes for recessive mutations are deaf, which may suggest the implication of another connexin gene eventually related to the formation of heteromeric connexons.

The GJB6 gene, encoding connexin 30 protein, is located in the same gene cluster as GJB2. Moreover, connexins 26 and 30 exhibit 77% identity and are expressed in the mouse cochlea. As such, GJB6 arose as a candidate for the mutations which would cause deafness in individuals with a single GJB2 mutation. However, some previous screening studies suggest that GJB6 point mutations are rare, especially when comparing with the high frequency of GJB2 mutations worldwide. Screening for GJB6 mutations in other populations should help to elucidate this puzzling situation.

In this study, we sought the contribution of the GJB6 gene for the aetiology of familial or sporadic deafness in the Portuguese population. The GJB6 gene was thus screened in hearing impaired individuals presenting monoallelic mutations or no mutations in GJB2

P0987. V84M variant in GJB2 gene associated with nonsyndromic autosomal dominant deafness

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Most pathogenic mutations in GJB2, which encodes the gap junction protein connexin 26, are recessive alleles associated with nonsyndromic prelingual hearing loss. Yet, a few alleles are described in the literature as being implicated in dominant forms of nonsyndromic deafness.

The present report describes a novel GJB2 variant, V84M, acting as a putative dominant allele. This variant was detected in two deaf patients, mother and daughter, both heterozygous for V84M. Identification was performed by SSCP followed by bidirectional sequencing and enzymatic restriction.

Variant V84M substitutes a valine for a methionine in the second transmembrane domain of the protein. The four transmembrane domains of connexins are highly conserved. Moreover, the valine at position 84 of connexin 26 is invariant across the connexins, thus enabling us to admit that its substitution probably leads to loss of function. So, V84M variant acting in a dominant negative manner might be the cause of deafness in this family. Another evidence that accounts for V84M being a putative dominant variant is the fact that the two carriers present a different degree of deafness, which is common in

cases of dominant deafness.

These data thus suggest that V84M might be a novel GJB2 mutation implicated in nonsyndromic autosomal dominant deafness.

P0988. Genetic testing in Czech patients with idiopathic and hereditary chronic pancreatitis.

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We have analyzed the entire *CFTR* gene coding region and screened for common *PRSS 1* (R122H, N29I), *SPINK 1* (N34S) mutations in a representative cohort of 65 Czech chronic pancreatitis (CP) patients comprising: idiopathic CP-iCP cases (inclusion criteria: *N Engl J Med* 1998, 339: 653) - 22 children / 32 adults and hereditary CP- hCP (EUROPAC inclusion criteria: *Med Clin North Am* 2000, 84: 575) - 7 children / 4 adults. Hundred random controls (50F, 50M; age range 18-45 years) were analyzed in parallel. In iCP the frequency of *CFTR* mutations F508del, R117H, L997F was increased only in adults ($p=0.01$) and was associated with an increase of the IVS8-9T / 10 TG haplotype ($p=0.001$) and of the 125G/C variant ($p=0.01$), compared to controls. The frequency of R122H and N29I *PRSS 1* mutations was significantly increased in both children/adults (3/22) with hCP ($p < 0.01$), but not in iCP cases (2/108) compared to controls. However, the *SPINK 1* N34S mutation was found at a considerable frequency also in controls (4/200 chromosomes). Our data indicate that mutations in *CFTR*, *PRSS 1* genes are associated with iCP / hCP, with *PRSS 1* alleles being more often common in "early onset" CP. *SPINK1* mutations may have a modifier role in the development of CP. In positive cases genetic counseling and long-term monitoring of eventual development of other cystic fibrosis-related symptoms (in iCP) and the adenocarcinoma of pancreas (in hCP) should be provided. Supported by MZCR: 000000064203.

P0989. Eight new mutations of the KIAA1985 gene associated with severe form of demyelinating autosomal recessive Charcot-Marie-Tooth disease (CMT4C) in 11 families and founder effects in families North African and European origin

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CMT is a pathologically and genetically heterogeneous group of hereditary motor and sensory neuropathies characterized by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished on anatomopathological and electrophysiological grounds: demyelinating and axonal CMT. Up to now, 8 different loci have been reported and 7 genes have been identified in demyelinating autosomal recessive forms of the disease. Recently, the KIAA1985 gene has been implicated in a severe demyelinating form of CMT associated with kyphoscoliosis.

We selected 38 consanguineous families with demyelinating ARCMT in whom 72 subjects were affected in a total of 178 individuals. These families were screened for 8 microsatellite markers covering the CMT4C locus. Assignment of the families to CMT4C was established by homozygosity mapping and confirmed by linkage analysis. The KIAA1985 gene was sequenced in all families with putative linkage to CMT4C.

We identified 11 families with linkage to the 5q32 locus. In 10 of them, eight new different mutations were identified. We also identified 2 mutations previously reported (Senderek et al., 2004), one of which was found in 5 of our 10 families. Together, both studies strongly suggest a common ancestor of these families. Indeed, this mutation was found in families originating from the Netherlands, Germany, Bosnia, North

Africa, Greece and Turkey. In agreement with this hypothesis, common haplotypes with flanking markers segregated in these families.

The genetic subtypes of AR-CMT linked to 5q32 locus account for 43% of our series.

P0990. Hereditary thrombophilia risk factors are not significantly associated with an increased risk of stillbirth in Czech patients

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The aim of this study was to assess the influence of inherited thrombophilia risk factors FV-Leiden (FV-L), FII 20210G->A (FII), MTHFR 677C->T (MTHFR) and PAI-1 4G/5G on the risk of stillbirth in the Czech population. In total, 52 cases with stillbirth (defined as a baby born with no signs of life after 24 weeks of gestation) and 102 healthy controls were genotyped. Only in FV-L there was a trend towards an increased risk of stillbirth in studied patients ($p=0.08$; odds ratio, 2.3). One of the patients was homozygous for FV-L (ns, odds ratio, 3.96). The frequencies of prothrombin FII and MTHFR mutations, including the PAI-1 4G/5G polymorphism were similar in patients versus controls (FII /2% vs. 2%/, for MTHFR /37% vs. 38%/ and PAI-1 4G/5G /56% vs. 58%/). Moreover, frequencies of combined genotypes of heterozygous FV-L mutation together with PAI-1 4G/4G hypofibrinolytic genotype in the same patient and heterozygous FII mutation with PAI-1 4G/4G allele were not statistically different from controls. We conclude that hereditary thrombophilia does not significantly influence the risk of stillbirth, even in combination of studied factors. Interestingly, despite pathogenetic heterogeneity of the sample under investigation FV-L was associated with a positive trend. Pregnant women could thus benefit from FV-L mutation screening and patients from anticoagulant therapy in subsequent pregnancies. Nevertheless, further larger scale studies are necessary. Supported by VZFMN 00000064203.

P0991. Identification of a novel splice variant produced by the Hermansky Pudlak Syndrome Type 1 Gene (HPS1)

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Hermansky-Pudlak Syndrome (HPS) [MIM#203300] is a rare, autosomal recessive disorder characterized by a triad of clinical features such as, oculocutaneous albinism, bleeding tendency caused by storage pool deficiency platelets, and a ceroid-like storage disorder. HPS exhibits locus heterogeneity, seven HPS-causing genes have been identified in humans. Patients with HPS type 1 are frequently found in the Northwest region of the island of Puerto Rico. Two major HPS1 mRNA variants result from alternative splicing of exon 9, encoding the major and minor protein isoforms of 79.3 and 75.9kDa, respectively. To date, four different splice HPS1 gene variants have been reported in GenBank. These splice variants were found to be expressed in human lymphoblasts by RT-PCR. The four mRNAHPS1 were detected in cells derived from a patient with HPS-1 and other tissues. Sequencing analysis of PCR amplification of the normal coding sequence (CDS) of the HPS1 mRNA showed an unexpected splice variant lacking exon 5. These studies show that the HPS1 gene expresses more than one transcript variant in human lymphoblasts. Further studies of these variants are necessary to better understand the role of each transcript in the pathways affected in HPS. Grants from NIGMS S06GM08224 and R25GM61838 and NCRRG12RR03051 supported this study.

P0992. Common Mutations In The Alpha-1-Antitrypsin Gene Are Not Frequently Found In Patients With Hermansky Pudlak Syndrome Complicated With Pulmonary Fibrosis

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The Hermansky-Pudlak Syndrome (HPS) [MIM #203300] is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding tendency, and a ceroid-lipofuscin-like lysosomal storage disease (Witkop et al. 1990). Frequent medical problems seen in HPS patients include prolonged bleeding, granulomatous colitis and progressive pulmonary fibrosis (PF). PF has been associated in 30-

40% of HPS cases. Deficiencies in Alpha-1-antitrypsin (AAT) produce a degenerative pulmonary disorder by a mutation in the gene of proteinase inhibitor 1 (PI). The S and Z alleles are the most common in persons manifesting AAT, while the M allele is the most common one in normal persons. The Z allele consists of a G->A in exon 5 (Lys 342->Glu), whereas the S allele is an A->T change in exon 3 of the AAT gene (Val 284->Glu). We utilized PCR combined with restriction digestion with TAQ a1 enzyme and DNA sequencing to screen for these AAT gene variants in PR HPS patients. Eighteen samples of HPS-1 and HPS-3 patients were examined for the exon 3 and exon 5 alleles of the AAT gene. All the patients examined for the exon 5 mutations had the normal M variant. Only two patients examined for exon 3 mutations had the mutation found in the S variant. One of the three patients with pulmonary fibrosis turned out to be heterozygous for the mutation of the S variant. The other heterozygote patient for this mutation has yet to show pulmonary problems. Grants from NIGMS S06GM08224 and R25GM161838 and NCRRG12RR03051 supported this study.

P0993. Sense and antisense Foxl2 transcripts in mouse

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FOXL2 is a forkhead transcription factor involved in the eyelid development and in the development and adult function of the ovary, in mammals. Its mutations in man are responsible for a rare genetic disease characterized by eyelid malformation, associated or not with premature ovarian failure (Blepharophimosis syndrome of type I and II, respectively. OMIM 110100). In mouse, we have identified 2 mRNA isoforms of Foxl2 that result from an alternative polyadenylation. Here, we characterize in depth the structure and expression of these 2 variants. We also describe an antisense transcript that overlaps the whole Foxl2 transcription unit. This antisense transcript, called Foxl2OS (for opposite strand), yields several isoforms resulting from alternative splicing. No significant coding region was found in the Foxl2OS sequence. Foxl2OS displays a pattern of expression very similar to that of Foxl2 in the gonads during development and at the adult age. RNA-FISH experiments show that both transcripts are expressed in the same cells at the same time. We suggest that Foxl2OS is a non-coding antisense RNA that may be involved in the regulation of Foxl2. All in all our results provide new insights about the organization of the murine Foxl2 locus. This might help understand its regulation and function.

P0994. Automated genomic DNA extraction: performance evaluations of the Abbott m1000™ system for multiple clinical diagnostic applications

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The m1000 instrument is a fully automated DNA extraction system based on chaotropic lysis of whole blood followed by capture and washing of DNA on magnetic particles and finally elution of DNA. Yield and purity are suitable for a wide range of molecular diagnostic applications. The system, developed for medium to high throughput workload, allows several different user-designed protocol options depending on the laboratory yield and concentration requirements.

Here, data are presented showing suitability of the genomic DNA system as exemplified for two widely used clinical applications: Cystic Fibrosis (CF) testing and HLA typing.

For CF testing 81 frozen blood samples were extracted and subsequent CF genotyping with a PCR/OLA assay revealed the expected normal/mutant types.

For HLA typing, where laboratories typically require good quality DNA in sufficient quantity for normal SSP, SSOP and/or SBT testing and also archiving, a large number of fresh and frozen blood samples (>8000) was extracted with successful HLA typing using this technique.

The m1000 was shown to meet requirements for rapid extraction of high quality DNA suited for genomic analysis. No sample cross-contamination was detected and the purified DNA was free of inhibitors. These data demonstrate the potential of the system to reliably provide suitable DNA in the molecular diagnostic setting generally.

P0995. Vitamin D Receptor Gene Polymorphisms in Turkish Psoriasis Patients

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Psoriasis is an inflammatory disease characterized with increased squamous cell proliferation and impaired differentiation. Although, vitamin D, Calcitriol, and its analogues are successfully used for psoriasis therapy, some psoriasis patients are resistant to Vitamin D therapy. Vitamin D mediates its activity by intracellular receptor. It's suggested that polymorphisms in VDR gene may explain the differences in response to vitamin D therapy.

In this study, 102 psoriasis patients and 102 healthy controls were studied for VDR gene polymorphisms. The Fok I, Bsm I and Apa I polymorphisms were examined by PCR-RFLP and 50 subjects received vitamin D therapy to evaluate the association between VDR gene polymorphisms and response to vitamin D therapy.

There was no significant difference between demographic data and clinical parameters of the patients and vitamin D therapy and VDR polymorphisms. However, Aa and bb genotypes were significantly higher in early onset than late onset psoriasis. The haplotype "FfBbAa" was significantly higher in patients than controls.

In conclusion, our results indicate that VDR gene polymorphisms does not affect the response to vitamin D therapy in psoriasis. However, "FfBbAa" haplotype and Apa I, Bsm I polymorphisms are associated with psoriasis and early onset of the disease, respectively.

P0996. Plasmid profiles of multidrug resistant uropathogenic *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.* and *Pseudomonas spp.* isolates from Nigeria

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Fifty-two multidrug resistant local uropathogenic *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, and *Pseudomonas spp.* isolates were tested for susceptibility to 10 antimicrobial agents. Tetracycline (100.0%) and ampicillin (94.2%) showed the highest rates of resistance, and ciprofloxacin (50.0%) demonstrated the lowest. The majority of MDR isolates showing concurrent resistance to all 10 agents (n=17) was a component of 32.7%. A total of 23 antimicrobial resistance patterns was observed. The predominant phenotype (32.7%) amongst the isolates included resistance to ciprofloxacin, cefuroxime, ceftriaxone, ampicillin, gentamicin, nalidixic acid, nitrofurantoin, co-trimoxazole, streptomycin, and tetracycline. A 38.5% (n=10) of the isolates were carrying plasmids of sizes estimated above 2.1kb. After curing, most of the mutant strains lost their plasmids as was corroborated by their improved susceptibilities. Given that uropathogens are demonstrating increasing antimicrobial resistance, epidemiological investigation using the molecular characterization tools is highly necessary.

P0997. Performance of a DHPLC based platform for comprehensive mutation detection in a clinical molecular diagnostic setting

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OBJECT: Denaturing High Performance Liquid Chromatography (DHPLC) is a sensitive, high throughput technique widely used in research to identify hundreds of mutations. We evaluated the effectiveness of DHPLC in a clinical molecular diagnostic setting.

METHOD: We designed 3 denaturing strategies to detect the common types of clinically important mutations: 1) using partial denaturing to form heteroduplex for rapid screening of base-pair deletions, insertions, and substitutions; 2) using complete denaturing to couple with primer extension to identify multiple target mutations simultaneously; and 3) using non-denaturing for straightforward quantitative detection of genomic deletions and duplications. We studied 518 previously tested clinical samples with documented abnormalities in the following genes: Dystrophin; SLC26A4; Factor V; Prothrombin; MTHFR; HFE;

and Mitochondrial genome. **RESULTS:** We correctly identified all of the genetic abnormalities including 37 exon/s deletions, duplications and 53 point mutations in Dystrophin gene; 16 SLC26A4 mutations; 29 homozygote, 35 double heterozygote, and 9 triple heterozygote in simultaneous detection of 3 targeted mutations associated with hypercoagulation (Factor V, Prothrombin, MTHFR) and 2 targeted polymorphisms related to hemochromatosis, respectively; and 62 mitochondrial mutations/polymorphisms. In addition, we found 12 new pathogenic mutations in these genes including point mutations, genomic deletions and duplications. These approaches, either single or combination, have significantly saved the time and cost of genetic tests. **CONCLUSION:** DHPLC offers a unique combination of sensitivity, accuracy, speed and cost-effectiveness in the detection of a variety of mutations. It is a platform that is highly suited to the clinical and regulatory environment of a diagnostic laboratory.

P0998. Four new mutations in the PINK1 gene in early onset autosomal recessive parkinsonism

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Parkinson's disease (PD) is a frequent disorder. A growing number of responsible loci/genes have been identified in familial forms. Mutations in the PTEN-induced kinase 1 (PINK1) gene located within the PARK6 locus on chromosome 1p35-p36 have recently been identified in autosomal recessive forms of PD. In this study we screened a large series of 145 families, of diverse geographic origins, with autosomal recessive parkinsonism and age at onset before 60 years, for PINK1 mutations.

After excluding 50 families with mutations in the parkin gene, 3 newly developed microsatellites markers flanking the PINK1 gene were analysed in the remaining 95. Sixteen familial cases presenting haploidentity and 5 cases with consanguinity presenting homozygosity at these markers were sequenced. Five new mutations were identified in the PINK1 gene in four families. Two homozygous substitutions in exon 6, G386A and G409V, and an homozygous deletion in exon 8, L519fs>522X, were identified in isolated patients with consanguinity. We also identified a compound heterozygous mutation (K24fs>54X and C549fs>553X) in the fourth family. The mutation in exon 1 is the first found in the 34-amino acid mitochondrial targeting motif. Patients had a phenotype of typical PD with early onset (range 22 to 52 years) with additional signs in those carrying the homozygous mutations. In conclusion, autosomal recessive mutations in PINK1 are a rare cause (2.8%) of young-onset Parkinson disease but might give new insight in the understanding of the disease.

P0999. Polymorphisms at the ligand-binding sites of the vitamin D receptor gene and osteomalacia

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Vitamin D receptor (VDR) gene polymorphisms have been suggested as possible determinants of bone mineral density (BMD) and calcium metabolism. In this study, our aim was to determine if there is an association between VDR gene polymorphism and osteomalacia. We determined Apal and Taql polymorphisms in the vitamin D receptor gene in 24 patients with osteomalacia and 25 age-matched healthy controls. Serum calcium, phosphorus, ALP, PTH, 25OHD levels were also examined. We used PCR and RFLP methods to test for an association between osteomalacia and polymorphisms within exon 8 and exon 9 of the VDR gene. After genetic analysis of the VDR gene, allelic variations were: AA, 50%; Aa, 45.8%; aa, 4.2% and TT, 33.3%; Tt, 62.5%; tt, 4.2% FF in patients and AA, 28%; Aa, 56%; aa, 16% and TT, 36%; Tt, 52%; tt, 12% in the healthy controls. When the

control and patients were compared for their Apal and Taql genotypes there was no relationship between VDR gene allelic polymorphisms and osteomalacia. Also no association between biochemical data and VDR gene polymorphisms was observed. Thus, we were unable to find an association between the VDR gene polymorphisms and osteomalacia.

P1000. The role of folates and/or cobalamine in occurrence and pathogenesis of trisomy 21

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Trisomy 21, the most frequent cause of mental retardation of genetic origin, is the most common of the chromosomal aberrations. It causes a characteristic syndrome of mental deficiency, malformations and neurological signs characteristic of Alzheimer disease. However these disorders are also observed in the event of deficiency in folates and cobalamines. These two vitamins play a capital part in the processes of methylation, the synthesis of DNA and the development of the foetus. Moreover, two genes of folate metabolism are localised on chromosome 21: CBS and RFC. Our hypothesis is that DS could be due to hypomethylation caused by a deficit of the enzymes implied in one carbon metabolism (MTHFR, MTR, MTRR, CBS and RFC). Moreover, hypomethylation leads to nondisjunction of the chromosomes during meiosis thereby playing a role in the occurrence of trisomy 21. We have studied the impact of these 2 vitamins in the occurrence of Trisomy 21 by examining various polymorphisms of genes involved in one carbon metabolism and by measuring biochemical factors (Homocysteine, B9, B12). Moreover, we studied, *in vitro*, on trisomic fibroblasts of patients in culture, the state of methylation according to the senescence and quantified the form of the folate carriers (RFC and FR).

P1001. 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 putative hormone which is secreted together with insulin by the pancreatic beta-cells. Several studies have implied a role for IAPP in bone remodelling. For example, adult mice heterozygote or homozygote for a null mutation in the IAPP gene have increased numbers of osteoclasts and develop an osteoporosis-like phenotype in adulthood. 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 are currently being processed and will be presented in further detail.

P1002. Associations and gene-environment interactions between single nucleotide polymorphisms (SNPs) in IL4 (c-589t) and IL4 receptor (I50V and Q576R) and total specific IgG to diphtheria and tetanus toxoids in children at risk of atopy

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Rationale: There is evidence for (1) high heritability for antibody, and

a Th2 cytokine (IL-13) responses to vaccines (2) atopy influencing immune system development, including delay in maturation from Th2 to Th1 bias and (3) an association between smoking and impaired vaccine response. To assess the role of these multi-factorial influences this study investigated the effects of: SNPs in the Th2 cytokine IL-4 and IL4 receptor; and parental smoking, on diphtheria and tetanus vaccine response in a cohort enriched for atopy. **Method:** The cohort was recruited at birth on the basis of a family history of atopy. Total specific IgG to diphtheria and tetanus toxoids at 2 years were measured by ELISA. Genotyping was performed by PCR and restriction digestion. **Results:** For the three SNPs studied (IL4 c-589t, IL4R I50V and IL4R Q576R) the allele previously associated with atopy was associated with increased antibody levels to diphtheria ($p=0.029$, $n=197$ $n=$; $p=0.062$, $n=191$ and $p=0.267$, $n=183$, respectively), and tetanus ($p=0.685$ $n=196$, $p=0.022$, $n=193$ and $p=0.055$, $n=185$, respectively) toxoids. Furthermore, for these genotypes interactions with smoking were found with diphtheria ($p=0.032$, $p=0.062$ and $p=0.079$, respectively) and tetanus ($p=0.001$, $p=0.022$, $p=0.067$, respectively) toxoid antibodies. Amongst those exposed to parental smoking "atopic alleles" were associated with increased vaccine responses, the converse being found amongst those without exposure to parental smoking. **Conclusion:** "Atopic alleles" of SNPs in IL-4 and IL-4 R are associated with increased antibody responses to diphtheria and tetanus toxoids, which is modified by parental smoking.

P1003. Novel autosomal recessive non-syndromic deafness locus maps on chromosome 6p21.2-22.3 in a large Tunisian consanguineous family

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Autosomal recessive nonsyndromic sensorineural deafness segregating in a large consanguineous Tunisian family was mapped to chromosome 6p21.2-22.3 defining a new locus. A maximum lod score of 5.36 at $\theta=0$ was obtained for the polymorphic microsatellite marker IR2/IR4. Haplotype analysis defined a 14.5 cM critical region between D6S1602 (50.75 cM) and D6S1665 (36.4 cM). This interval overlap with DFNA13, DFNA21 and DFNA31 loci. The entire coding region of COL11A2, responsible of the DFNA13 deafness, was analysed by conformation sensitive gel electrophoresis (CSGE) and sequencing analysis. No mutation was observed. The genes corresponding to the ESTs mapped to chromosome 6p21.2-22.3 are being screened for deafness-causing mutations.

P1004. Results of a genetic scan of the X-chromosome and implications for the genetics of synaesthesia

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Synaesthesia, a neurological condition affecting approximately 0.05% of the population, is characterised by anomalous sensory perception: a stimulus in one sensory modality triggers an automatic, instantaneous, consistent response in another modality (e.g. sound evokes colour) or in a different aspect of the same modality (e.g. black text evokes colour). Growing evidence links synaesthesia to cognitive dysfunction; dyslexia, dyscalculia and cognitive interference from synaesthetic experiences have been reported. Conversely, anecdotal and experimental evidence has linked synaesthesia to enhanced recall and absolute musical pitch.

Family studies have shown evidence of a strong underlying genetic predisposition with 48% prevalence among first-degree relatives of synaesthetes and greater risk to female than to male relatives. Pedigree analysis and evidence from previous studies suggests an X-linked dominant mode of inheritance. The results of a genetic linkage

scan of the X-chromosome using 20 microsatellite markers (average inter-marker spacing 10cM) in 27 multiplex families will be reported. Additionally, following an investigation of two cases of male-to-male transmission of synaesthesia raising the possibility of oligogenic inheritance, a whole genome screen using 400 microsatellite markers was commenced. Progress to date will be reported.

P1005. Haplotype analysis of the serotonin transporter gene in Russian suicide attempts

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Genetically-mediated alterations in serotonergic transmission have been implicated in suicidal behavior. Recent studies showed that sex differences exist in the contribution of genetic factors to suicidal behaviour. The aim of this study was to estimate the contribution of the serotonin transporter gene (*SLC6A4*) in suicidal behaviour for males and females of Russian origin. A total of 100 suicide attempts (66 females and 34 males) were included in this study. The control group included 167 individuals (58 females and 109 males) without a personal/family history of any psychiatric disorders. Two polymorphisms in the *SLC6A4* gene - 5-HTLPR (in the promoter region) and VNTR (in the second intron) were analyzed using PCR technique. Maximum likelihood analysis of haplotype distribution demonstrated the presence of linkage disequilibrium between the two polymorphic in control subjects (females $\chi^2 = 32.25$, $p < .00001$; males $\chi^2 = 32.93$, $p < .00001$), in suicide attempts (females $\chi^2 = 15.42$, $p = .0004$; males $\chi^2 = 15.42$, $p = .078$), in the overall samples (females $\chi^2 = 39.24$, $p < .00001$; males $\chi^2 = 26.33$, $p < .00001$). Analysis of distribution of the estimated haplotype frequencies revealed a significant difference between suicide attempts and control subjects in females only ($\chi^2 = 15.52$, $df = 5$, $p = .002$). The haplotype S12 had lower frequency in female suicide group ($\chi^2 = 6.11$, $df = 2$, $p = .014$, OR = 0.49, 95%CI = 0.28-0.87). Our findings indicate the contribution of the *SLC6A4* gene to susceptibility for suicidal behaviour in females only.

P1006. Hypoxia-inducible factor-1 (HIF-1) gene polymorphism as an ischaemic stroke risk factor in Moscow population

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Hypoxia-inducible factor-1 (HIF-1) belong to the basic-helix-loop-helix (bHLH)-PAS protein superfamily. HIF-1 is a heterodimeric transcription factor consisting of HIF-1 α and HIF-1 β subunits. The expression and the level of the HIF-1 α is regulated by protein stabilization under hypoxic and protein degradation under normoxic conditions. HIF-1 α protein levels increase in cells to hypoxia. Under low O₂ tensions, HIF-1 α enhance the expression of genes encoding erythropoietin (EPO), vascular endothelial growth factor (VEGF) and some other. Thus, the aim of our investigation was to study the connection between IVS9-675C>A polymorphism in gene HIF-1 and atherothrombotic ischaemic stroke. We enrolled 60 patients with atherothrombotic ischaemic stroke from Moscow and controls group consisting of 104 healthy persons. Polymorphism of HIF-1 gene was studied by PCR and restriction SmiM1. The A allele frequency was higher in controls group (88.46%) than in patients group (80.83%) and the frequency of C allele was higher in patients group by 7.63%. C/C genotype wasn't found in patients group although its frequency was 3.85% in controls. This differences was statistically significant ($p = 0.0016$ for genotype frequency, 0.043 for allele frequency). Thus, the patients with C/C and C/A genotypes have higher rate of development of stroke than ones with A/A genotype (OR = 1.99; CI [1.92; 2.03]). Also we analyzed a possible association between HIF-1 α gene polymorphism and stroke severity. But it was not found significant correlations between IVS9-675C>A polymorphism of gene HIF-1 and brain infarction volume on 1, 3, 7, 21 days after stroke and stroke severity according to Orgogozo scale.

P1007. Genetic polymorphisms in the CYP1A1, CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1 genes and their relationship to chronic bronchitis and relapsing pneumonia in children.

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Aim of this study was to investigate the possible roles of the genes functioning in xenobiotic metabolism and antioxidant pathways in the development of severe chronic lung disease in children.

Polymorphisms in the genes encoding CYP1A1, CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1 were investigated in Tatar children with chronic lung disease (CLD): chronic bronchitis (N=129) and relapsing pneumonia (N=50) and in ethnically matched healthy individuals (N=227) living in Ufa, Bashkortostan by PCR-RLFP method.

The frequencies of *2C allele of CYP1A1 gene were significantly higher in CLD patients than in the healthy control group ($\chi^2=15.02$, $P=0.0007$).

This allele were associated with higher risk of chronic bronchitis in children (OR=4.14 CI 95% 1.83-9.53). Similar results were obtained in the relapsing pneumonia patients (OR =3.86 CI 95% 1.34-10.95 for *2C allele). The patients with CLD showed significantly elevated frequencies of the GSTT1 gene deletion ($\chi^2=10.72$ $P =0.0019$). The increase of the GSTT1 gene deletion was significant only in the case of chronic bronchitis (OR=2.44 95% CI 1.48-4.04).

The distribution of the EPHX1, CYP2E1, GSTM1 and GSTP1 gene genotypes did not significantly differ between CLD patients and healthy subjects.

Our findings support that the polymorphisms of the CYP1A1 and GSTT1 genes, which code for enzymes with dramatically altered activities, probably play a substantial part in susceptibility to severe pulmonary inflammation in children with CLD.

P1008. Genetic Heterogeneity of PKD1 & PKD2 Genes In Iran And Determining The Genotype/Phenotype Correlations In Several Families With Autosomal Dominant Polycystic Kidney Disease

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic nephropathy. So far, three genetic loci have been identified to be responsible for ADPKD. Little information is available concerning the pattern of linkage in Iranian population. In the present study the linkage analysis was performed using three pairs of polymorphic microsatellite markers including 16AC2.5-CA (D16S291), SM7-CA (D16S283) and KG8-CA (intragenic marker at the 3' end of the gene), that were closely linked to the ADPKD1 locus and three pairs of selected polymorphic microsatellite markers including YUNCA9 (D4S231), AFM155xe11 (D4S1534) and AFM224x6 (D4S423) which were closely linked to the ADPKD2 locus. In parallel, the genomic DNA of 150 unrelated healthy individuals were used to determine frequency, heterozygosity rate and PIC for each marker. Assignment of the disease gene loci was performed following phasing and haplotype construction, genotype/phenotype correlations were deduced from the constructed haplotypes. Our results showed relatively high heterozygosity rates and PIC values for some markers, while the most informative markers were KG8 and 16AC2.5 for *PKD1* gene and AFM224x6 for *PKD2* gene. We report here the first molecular genetic study of ADPKD and the existence of locus heterogeneity for ADPKD in Iranian population by performing linkage analysis on 15 affected families. Eleven families showed linkage to *PKD1* and two families linked to *PKD2* gene. In 2 families, *PKD1* markers were common in all affected members but *PKD2* markers were not informative. In our study, a gene frequency of 0.001 was assumed for *PKD1* and 0.0001 for *PKD2*.

P1009. Association of the MCP-1 promoter polymorphism with tuberculosis in the Hong Kong Chinese population

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Hong Kong is a place of intermediate burden of tuberculosis (TB). The TB notification rate in Hong Kong was 108 per 100,000 persons in 2001. MCP-1 is a chemokines recruiting activated T cells to form granuloma in mouse *M. tuberculosis* (MTB) infection, suggesting MCP-1 involved in the immunopathogenesis of TB. A functional polymorphism (A-2518G) in the promoter region of MCP-1 was shown to influence the transcriptional level of the gene. We hypothesize that MCP-1 polymorphisms may contribute to human susceptibility to TB. Genomic DNA was extracted from frozen EDTA-treated whole blood samples of TB patients and blood donors. A 930 base pair segment of the MCP-1 were amplified by polymerase chain reaction (PCR) and the genomic variants were detected by restriction fragment length polymorphism (RFLP) using restriction enzyme, Pvull. We performed a population case-control study to test if the -2518 A/G polymorphism of the MCP-1 gene contributes to the susceptibility to tuberculosis. A total of 941 individuals, including 447 controls and 494 TB patients, was genotyped. The GG genotype and the G allele of the MCP-1 gene polymorphism were significantly more common in the TB patients than in the controls ($P = 0.01$). This finding indicates that the -2518 A/G polymorphism of the MCP-1 gene is associated with TB in Hong Kong Chinese population.

P1010. Variability in apo(a) gene control regions and correlation to Lp(a) plasma levels

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High lipoprotein(a)[Lp(a)] level is an independent risk factor for development of premature atherosclerosis. Apolipoprotein(a)[apo(a)] is the main determinant of Lp(a) plasma concentration. The aim of our study was to assign if particular variants of apo(a) gene control regions (promoter, DHII, DHIII enhancers) or their combinations are correlated to specific Lp(a) levels.

Randomly chosen individuals were sorted out into several groups according to Lp(a) level. The relevant control regions were scanned for polymorphism occurrence using the DGGE method, fragmentation analyses and sequencing. Population frequencies of polymorphic variants were obtained from a sample of 263 individuals by means of the RFLP assay and the PCR amplification of specific alleles method. The linkage disequilibrium was measured by RelD and Δ statistic.

The DHII enhancer is fully conserved. As we expected, the promoter site revealed only a restricted variability. The most polymorphic region was shown to be the DHIII enhancer, which discloses several polymorphisms as well as relatively high frequency of mutations. We detected strong linkage disequilibrium among proximal and distal promoter regions and DHIII enhancer sites. Finally, some polymorphic variants have distinct frequencies among groups with a different range of Lp(a) level.

We conclude from our study that variability in apo(a) gene control regions assemble complex haplotypes which could be connected with specific range of Lp(a) levels.

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P1011. Primary ciliary dyskinesia (PCD): Identification of a novel locus in the Arabic population with atypical central pair transposition defect

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PCD is a rare autosomal recessive disorder characterised by respiratory tract infections and subfertility. The clinical phenotype results from dysmotility of the cilia, which is associated with a variety of structural abnormalities. The core or axoneme of cilia comprises a bundle of microtubules and associated proteins including dyneins, nexin links and radial spokes. About 50% of patients exhibit laterality defects, commonly *situs inversus*, association known as Kartagener syndrome. We have studied large consanguineous family from UAE. Parents are first cousins with three affected children (None of whom have *situs inversus*) and eight unaffected individuals. They have circular ciliary beat pattern which is consistent with atypical ciliary transposition defect. A genomewide scan identified a region consistent with linkage on 6p21.2. Using GENEHUNTER, a maximum multipoint LOD score of 2.9 was obtained between novel microsatellite C6orf197-CA and D6S282. This critical region spans approximately 6 megabases of genomic DNA. Work is in progress to using comparative genomics approach to identify potential candidate genes. There are 40 known genes in this region of which two have been identified as potential candidates. *DNAH8*, axonemal heavy dynein8, is an integral component of cilium. *DNAH5* (protein from same gene family) knock out mice exhibit phenotype similar to that of human PCD. Another candidate *KNSL8*, kinesin light chain8, is essential for intraflagellar transport of ciliary components. Both genes are expressed in the lungs and testes. *DNAH8* and *KNSL8*, therefore represents an excellent candidate gene for PCD. There genomic characterisation will be undertaken before performing mutational analysis in patients.

P1012. Association of B lymphocyte stimulator (BLyS) polymorphisms with systemic lupus erythematosus (SLE)

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Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by dysregulation of T and B lymphocytes, leading to production of autoantibodies and immune complexes (ICs). Recently, a promoter polymorphism of B Lymphocyte stimulator (BLyS, also known as BAFF, TALL-1, zTNF4, TNFSF13B) was found to be associated with higher anti-Sm antibody level in Japanese. BLyS promotes B cell differentiation, proliferation and survival. It is located in chromosome region 13q32, which is a susceptibility locus of SLE. In BLyS-/- mice, number of B cells, serum IgG and IgM levels were decreased and B cell development was blocked at transitional T1 stage. Higher BLyS level was found in SLE patients. Therefore, we hypothesized polymorphisms of BLyS may affect the susceptibility and development of clinical features of SLE in our population.

Association of 4 promoter single nucleotide polymorphisms (SNPs) (-1283G/A, -871C/T, -514T/C, -353G/C) and 1 intronic SNP (IVS1-45C/G) of BLyS were analyzed in 456 SLE patients and 760 healthy controls, using high-throughput Sequenom Assay.

No significant association was found in the promoter SNPs with disease susceptibility. However, frequency of G-carrier of the intronic SNP IVS1-45C/G was found to be higher in the controls ($P = 0.03$). In addition, -871CC was over-represented in patients with Anti-nRNP ($P = 0.0046$; OR 2.38; 95% CI 1.33-4.23).

This suggests that the allele G of the intronic SNP IVS1-45C/G of BLyS may increase the risk for developing SLE, while -871CC is associated with the development of Anti-nRNP in SLE patients.

P1013. SPG7 in 139 patients with Hereditary Spastic Paraparesis

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Mutations in the SPG7 gene, which encodes paraplegin, are responsible for an autosomal recessive form of pure or complicated hereditary spastic paraparesia (HSP). All 17 exons of SPG7 were analyzed using DHPLC and/or direct sequencing in 139 probands with pure or complicated HSP and autosomal recessive inheritance, 41 with consanguinity.

We found 50 different heterozygous sequence changes, 40 of which were not previously reported. Twenty-six of these variants were found in 22 families and were absent in controls, 18 of them in coding regions. No homozygous mutations were identified, even in the 7 consanguineous families, but 5 out of 22 families had 2 heterozygous changes.

Two families with complicated HSP with cerebellar signs and onset at age 27 and 28, had mutations affecting the protein sequence: 850-851 delTTinsC and V581del in a Moroccan family and R294H and N730D in a Mauritanian family.

Two other families had the same association of a missense mutation (A2T) and a synonymous mutation (L67L) located in cis. One family associated 2 intronic variations and one synonymous mutation of unknown effect. We also identified 17 families with only one heterozygous mutation, including 4 with highly probable pathogenic mutations (M1L, IVS11-1-1457 del9, Q507X, Q82del).

In conclusion, mutations in the paraplegin gene are rare and represent < 2% (2/139) of the families studied. The frequency of rare nucleotide variants was high, however, complicating routine diagnosis.

P1014. Association of CTLA-4 Gene Polymorphisms with Coeliac Disease in the Maltese Population

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Coeliac disease (CD) has an autoimmune component in genetically predisposed individuals triggered by environmental factor (gluten). The disease manifests in partial or total villous destruction of the small intestine with malabsorption and malnutrition. The main environmental triggering factor is a transglutaminated peptide within the gliadin component of gluten, found in wheat. CD has an established HLA component responsible to 35% of the genetic predisposition, rest being in the non-HLA region.

100 coeliac patients were recruited, having predominance of females over male coeliac patients (3:1, $\chi^2 = 25$, $p < 0.001$). The mean age at diagnosis for the whole group was 34 years (males 32 years, females 34 years, $t = -0.65$, N.S.). The predominant presenting symptoms were gastrointestinal related. A higher proportion of males reported a positive family history as compared to females ($\chi^2 = 5.44$, $p = <0.02$). Two polymorphisms found within the CTLA4 gene were studied amongst a sample of coeliac patients and cord blood DNA samples ($n = 187$) that acted as the control group. Polymorphisms within the CTLA4 gene have been associated with other autoimmune conditions and the gene plays a very important role in immunoregulatory function. The coeliac individuals and cord blood samples were genotyped for the -318 C/T and +49 A/G SNPs. No association of the single polymorphisms or the combined haplotypes with the coeliac condition was apparent amongst the coeliac patients under study. The -318 C allele and the +49 A allele were in linkage disequilibrium amongst the cord blood samples.

P1015. Molecular-genetics analyses of hepatolenticular degeneration in Republic of Moldova.

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Hepatolenticular degeneration also known as Wilson Disease (WD) is an autosomal recessive inherited disorder of copper metabolism, characterized by excessive accumulation of copper in the liver, central nervous system, kidneys, eyes and other organs, in individuals ranging in age from 4 to over 50 years.

Our 28 patients had presented with predominantly hepatic, neurological or psychiatric manifestations. 15 of them were children who have one of liver diseases that clinically manifests at the age of 4-8 years. Neurological and psychiatric findings manifest in 48 % of the patients. These patients are characterized by combination of tremor, dystonia, dysarthria, dysphagia, chorea, drooling, open-mouthedness, parkinsonian symptoms - rigidity and bradykinesia and incoordination. Inappropriate behavior sudden changes, difficulty concentrating, deterioration of schoolwork, emotional lability was noted in about 30 % of the patients. The molecular genetic analysis of the linkage loci D13S118 and D13S228 of the ATP7B gene responsible for WD among the 41 and 20 patients respectively, allowed to observe a heterozygosity of 0,59 (for D13S118) and of 0,80 (for D13S228). Comparing our results with the frequencies from the GDB we established that in our population the 189 bp., 193 bp. allele are more frequent and 195 bp is less frequent then in the European population for D13S118 loci.

The complex clinical neurological and molecular genetic methods applied in our study for WD turned out to be very efficient in establishing a correct diagnosis and prescribe a correct treatment, if necessary.

P1016. Xenobiotics-metabolizing enzymes genes and cancer: a link between polymorphisms and environment

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Polymorphisms of *CYP2E1*, *CYP2C19*, *GSTT1*, *GSTM1*, *GSTP1* genes were studied in 287 breast cancer (BC), 102 lung cancer (LC), 72 head and neck cancers patients from Tomsk and in 96 workers of the largest Russian nuclear energy combine - Siberian Group of Chemical Enterprises (SGCE) with different cancer types. For control, 221 healthy people from Tomsk and 102 SGCE workers were used.

We found, that "slow" *CYP2C19*2* allele was a risk factor for LC and BC in Tomsk: its frequency in patients was twofold higher than in controls (37.3%, 32.9% and 17.0%, respectively, in LC, BC, and controls; $p<0.001$). Also, *GSTT1* "null"-allele homozygosity was associated with LC (40.5 % and 23.1 % in LC and controls, respectively; $p=0.005$).

In SGCE cancer cases there was an excess of the "fast" *CYP2C19*1* allele in comparison with controls (84.9% and 65.1%, respectively; $p=3.6E-4$) and an excess of *GSTM1* "null"-allele homozygotes (63.7% and 39.5%, respectively; $p=9.7E-3$). Such genetic features should, in theory, correspond to relatively fast accumulation and slow degradation of potential carcinogens. At that, there were no differences between frequencies of these alleles and genotypes in SGCE cancer cases and healthy people from Tomsk, suggesting that not yet identified carcinogen is present at SGCE and it has more significant etiological potential in carriers of adverse combination of *CYP2C19* and *GSTM1* alleles.

Thus, the common polymorphism of *CYP2C19*, *GSTT1*, and *GSTM1* genes is the risk factor for different cancer types, but its negative phenotypical effects significantly depend on the environment (carcinogens and modifiers presence).

P1017. Mutational analysis of genes BBS1 and BBS6 involved in Bardet-Biedl syndrome in Spanish patients

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Bardet-Biedl syndrome (BBS, MIM209900) is a clinical and genetically heterogeneous disorder. BBS patients manifest a complex and

variable phenotype that includes as cardinal characteristics: retinal dystrophy, polydactyly, mental delay, obesity and hypogonadism; other characteristics are renal dysplasia, dental malformations and a specific facies with wide forehead, hypoplasia malar, depressed eyes, large nose, fine uplip, everted down-lip, and retrognathia in lateral vision; additional features such as asthma, situs inversus and diabetes mellitus may also be present. Although BBS is rare in the general population there is a considerable interest in identifying the genes causing BBS because components of the phenotype, such as obesity and diabetes, are very common. The prevalence varies from 1/13.500 among Bedouin population to 1/160.000 in Western Europe. Until now eight BBS loci have been described: BBS1 on 11q13, BBS2 on 16q21, BBS3 on 3p11, BBS4 on 15q22.2-q23, BBS5 on 2q31, and BBS6 on 20p12, BBS7 on 4q27 and BBS8 on 14q32, with evidence for at least one more locus. Recently the theory of the segregation of this disease in an autosomal recessive manner was eclipsed by a new one that presents this segregation as a triallelic inheritance. This suggests that multiple alleles may act in concert to cause pathogenesis. We analyzed by PCR-SSCP the involvement of mutation in genes BBS1 and BBS6 in 43 patients. Several changes have been observed in both genes. Our results indicated that mutation M390R in BBS1 is the most frequent mutation founded appearing in 12% of the disease alleles.

P1018. Exclusion of GDAP1 and MTMR2 genes responsible for CMT4A and CMT4B1 phenotypes in autosomal recessive Charcot-Marie-Tooth Disease (AR-CMT).

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Charcot-Marie-Tooth diseases are a genetically heterogeneous group of disorders with autosomal dominant (AD), X-linked recessive and autosomal recessive (AR) inheritance. AD form is the most common one. There is progressive muscular weakness and atrophy affecting the distal extremities in all types. AR-CMT is less common and clinical manifestations are usually similar to AD-CMT; however, generally more severe and has an earlier age of onset. Electrophysiological classification as demyelinating or axonal is generally valid. AR-CMT's, are also genetically heterogeneous. More than 11 chromosomal loci have been assigned so far. We hereby report two large Turkish families originating from the same village in central Anatolia with CMT. Entire pedigrees structures of the first and second families consisted of a total of 46 and 18 individuals (7 and 4 affected members) respectively. More than one affected members in the same generation and highly inbred nature of the pedigrees suggested an autosomal recessive inheritance potentially originating from a common ancestor. Electrophysiological data of the two affected individuals confirmed demyelinating CMT. Genetic linkage analysis of all reported loci has been initiated and the candidate regions on 8q13 (GDAP1) and 11q22 (MTMR2) have now been defined. The respective order of the DNA markers and candidate genes are: D8S279-(1.26Mb)-D8S2324-(1.01Mb)-GDAP1-(0.4Mb)-D8S548-(1.9Mb)-D8S541 and D11S4176-(1.49Mb)-D11S1757-(0.87Mb)-MTMR2-(2.25MB)-D11S1390. We used an initial screening panel containing a total of 12 informative offspring (7 affected) from both families. Neither a common haplotype segregating in autosomal recessive fashion nor a significant lod scores was observed in both regions. Linkage analysis of the remaining loci are currently underway.

P1019. Spectrum of germ-line MLH1 and MSH2 mutations in Austrian Hereditary Nonpolyposis Colorectal Cancer patients.

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Background: Germ-line mutations in mismatch repair genes are associated with the hereditary nonpolyposis colorectal cancer

syndrome. The latter is characterized by a susceptibility to cancer of the colon, endometrium, small bowel or urothelium at an unusually young age and with a high degree of penetration in all generations.

Material and Methods: We analyzed 109 individuals from 46 Austrian families, who fulfilled the Amsterdam criteria (n=29) or at least one of the Bethesda guidelines (n=17), for mutations in *MLH1* and *MSH2*. Microsatellite instability was determined in the tumors of the index persons and affected relatives.

Results and Conclusion: High-grade instability was present in 60.6% of the tumor samples from index patients. Twenty-three germ-line DNA sequence variants in 24/46 families and 4 somatic mutations in 3 tumors were detected in *MLH1* and *MSH2*. Fifteen mutations are novel. None of the newly identified germ-line variants were found in 100 alleles of healthy control individuals. We were able to characterize two intronic variants (*MLH1* c.589-10T>A; *MSH2* c.367-1G>A) with regard to their effect on mRNA. Both created new splice sites that replaced the regular ones. Germ-line mutations occurred in 44.8% of the families fulfilling the Amsterdam criteria and in 35.3% of the Bethesda patients. The detection of a pathogenic mutation was highly significantly correlated with microsatellite instability in the tumor DNA ($p=0.007$). This study is the first comprehensive report of mismatch repair gene mutations in Austrian HNPCC patients.

P1020. Polymorphism of SLAM gene in RA in French population

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Rheumatoid arthritis (RA) is a complex genetic disease where numerous genetic factors contribute to susceptibility. CD150 signaling lymphocytic activation molecule (SLAM) is a cell surface glycoprotein found on activated B cells, T cells and dendritic cells.

In order to investigate the role of SLAM gene in the susceptibility to RA, we have analysed three SNPs (*rs 2295612*, *rs 1809963* and *rs 3796504*) localised in exon 1, in intron 4 and in exon 7 respectively, using PCR-RFLP technique. Statistical analysis was performed by Transmission Disequilibrium Test (TDT).

One hundred French caucasian trio families composed of one affected subject and the two parents were investigated. The mean age at disease onset was 39.6 years.

Our analysis showed no significant association with RA susceptibility ($p=0.3079$ for *rs 2295612*; $p=0.314$ for *rs 1809963* and $p=0.744$ for *rs 3796504*).

The lack of association of SLAM gene polymorphism in our study suggests that the SLAM gene is not a candidate gene for RA predisposition.

P1021. Association of VDR gene with thyroid autoimmune diseases in Tunisian population

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Autoimmune thyroid diseases (AITDs) including Graves' disease (GD) and Hashimoto's thyroiditis (HT) are inherited as complex traits. In order to detect the susceptibility genes involved in the pathogenesis of these diseases, we analysed the role of vitamin D receptor (VDR) gene polymorphisms (*FokI*, *BsmI* and *TaqI*) localised in exon 2, intron 8 and exon 9.

Our study concerned one hundred Tunisian patients affected with AITDs subdivided into 55 patients affected with GD and 45 affected with HT and one hundred unrelated healthy subjects. Molecular genotyping was performed by PCR-RFLP technique and statistical analysis was performed using *Chi 2X2* test.

Our results showed significant association of *BsmI* 'b' allele and 'bb' genotype with autoimmune thyroid diseases ($p=2 \cdot 10^{-4}$ and $p=7.8 \cdot 10^{-6}$) respectively. However, no significant association of *FokI* and *TaqI* polymorphisms was found between patients and controls ($p=0.328$ and $p=0.13$) respectively.

These results suggest that VDR 'b' allele and 'bb' genotype have a potential effect in AITDs.

P1022. Autozygosity mapping reveals deletion of MCPH1 gene as cause of autosomal recessive mental retardation in consanguineous Iranian family

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Autozygosity mapping in 6 affected individuals (4 boys and 2 girls) and their healthy parents of a large Iranian family with severe mental retardation (MR) and microcephaly has enabled us to map the underlying gene defect to the short arm of chromosome 8. Linkage analyses yielded a single peak at 8p22-p23.2, with LOD scores of 25 and 4.2, respectively, upon non-parametric and parametric multipoint linkage analysis using Merlin and GeneHunter. Thorough examination of the linkage data and haplotypes revealed an abnormal pattern of inheritance for several markers in the middle of this peak, and analysis of the raw genotyping data identified a 50-480 kb deletion encompassing three SNP markers as well as part or all of the MCPH1 gene.

MCPH1 is one out of only three genes for autosomal recessive MR that have been identified to date, and it has been shown to play a role in chromosome condensation. So far, only two point mutations in this gene have been described, and thus, the deletion mutant found in the Iranian family is the first of its kind. Cytogenetic analysis of these patients showed high number (10%-15%) of prophase-like cells in routine preparations and poor-quality metaphase G-banding, and clinically, the patients in this family are also indistinguishable from previously reported patients. Thus, our findings demonstrate that contrary to earlier speculations, MCPH1 null mutations are not lethal. High-resolution array CGH experiments are in progress to precisely define the borders of this deletion.

P1023. Two large French families with late-onset focal dystonia not linked to the three known loci DYT6, DYT7 and DYT13

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Focal Idiopathic Torsion Dystonia (FITD) is a group of movement disorders which is usually autosomal dominant with reduced penetrance. Commonly described forms of FITD include cervical dystonia (spasmodic torticollis), blepharospasm, oromandibular dystonia, laryngeal dystonia (spasmodic dysphonia), and limb dystonia (among which task-specific dystonias as writer's cramp). We studied two large French families presenting with varied symptoms of adult-onset FITD: spasmodic torticollis, postural tremor, blepharospasm, "task-specific" cramp and dysphonia. The first family, DYST143, is composed of 26 subjects (five definitely affected, two with unclear status). The average of onset is 43 ± 20 years. The second family, DYST154, included 21 subjects (three definitely affected, two with unclear status). The average of onset is 32 ± 16 years. DYST143 and DYST154 were studied for the three loci known to be implicated in FITD, DYT6, DYT7 and DYT13, by genotyping all individuals with markers spanning these regions: **DYT6** (Tel-D8S1791-D8S532-D8S587-D8S507-D8S1178-D8S1797-D8S1775-D8S279-D8S1475-Cen), **DYT7** (Tel-D18S59-D18S481-D18S54-D18S1154-D18S63-D18S452-D18S1163-D18S464-D18S53-Cen), **DYT13** (Tel-D1S2663-D1S450-D1S2667-D1S228-D1S507-D1S2697-Cen). Haplotype construction showed no common haplotype shared among all definitely affected family members within the 3 regions. These results were confirmed by Lod scores < -2 for DYST143. For DYST154, non informative Lod scores for DYT7 were obtained, but the implication of this locus would lead to a change of status for three subjects currently aged of more than 32 years old and scored as unaffected. **Conclusion:** These results illustrate the great genetic heterogeneity of FITD and indicate the existence of one or more unassigned genes for this pathology in the French population.

P1024. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease

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Recent data suggest that variants of the organic cation transport genes OCTN1 (a missense substitution in exon 9) and OCTN2 (a G-C transversion in the promoter) may represent disease causing mutations which account for the genetic contribution of the IBD5 locus, on chromosome 5q31, to the development of Crohn's disease (CD) (Peltekova *et al*(2004) *Nature Genetics* 36:471-475).

We have genotyped 374 CD, 305 ulcerative colitis (UC) and 294 healthy controls (HC) for five SNPs in the IBD5 region, including the published OCTN1 and OCTN2 variants. Association with disease susceptibility and phenotype was determined in CD and UC patients. We have confirmed the findings of Peltekova *et al*, that the variants of OCTN1 and OCTN2 are associated with CD but not with UC, and that the TC haplotype of OCTN1/OCT2 is associated with CD (25.3% v 16%, P=0.0035) when compared to HC. We have also shown that this haplotype is associated with disease progression (P=0.049) and disease severity, as measured by the need for surgery in CD patients (P=0.004). All five SNPs were in strong linkage disequilibrium: in the absence of the IBD5 risk haplotype we do not see an association of OCTN1/OCTN2 variants with CD.

Analysis was also performed to look for epistatic interactions between the IBD5 SNPs and Nod2 variants but no evidence of interactions were found.

P1025. Molecular characterization of nonsyndromic craniosynostosis

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Nonsyndromic craniosynostosis (NSC) is a heterogeneous condition of multifactorial etiology with evidence of genetic factors that are yet to be identified. Hot-spot mutation analysis for FGFR1 exon IIIa, FGFR2 exons IIIa and IIIc, FGFR3 exon IIIa, and TWIST1 was performed on 147 patients with presumed NSC. Two TWIST1 and two FGFR3 P250R mutations were identified among 21 patients with coronal synostosis. Another FGFR3 P250R mutation was identified in a patient with cloverleaf skull, indicating the extreme variability of Muenke syndrome. Twenty patients with isolated sagittal NSC were analyzed for mutations in the entire coding sequence of FGFR1, 2, and 3, FGFR1, SNAIL, SLUG, TWIST1 and 2, MSX2, RUNX2, and NELL1. These genes were selected on the basis of their biologic function and/or involvement in similar phenotypes. No obvious disease causing mutations have been identified so far. Familial nonsynonymous SNPs were identified in RUNX2, NELL1, and TWIST1 in several NSC families and are being evaluated as disease predisposing variants. The entire TWIST2 gene was sequenced for 19 patients with coronal NSC, 17 patients with metopic NSC, and 9 patients with lambdoid NSC, but no mutations were found. The negative results of the sequencing analysis of these candidate genes demonstrate that more efficient strategies, such as association analysis, are needed for the identification of genes contributing to NSC. In a study of 34 trios with sagittal NSC using 1048 SNPs in 82 craniofacial genes we established an association to chromosome 3q12, which is currently being validated with additional families.

P1026. Association of polymorphic variants of human NRAMP1, VDR, IL12B, IL1B and IL1RN genes with tuberculosis and clinical traits of the disease

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To analyze associations between genetic markers and tuberculosis (TB) and its clinical traits in ethnically homogenous population, common polymorphisms of five TB candidate genes were studied: NRAMP1 (469+14G/C, D543N, 1465-85G/A, 274C/T), VDR (F/f, B/b), IL12B (1188A/C), IL1B (+3953A1/A2) and IL1RN (VNTR in second intron) in 240 TB patients and 263 controls from Tuva Republic. Only 1465-85G/A polymorphism of NRAMP1 gene was associated with TB itself and with infiltrative form of the disease (p<0.05), suggesting just a little impact of the investigated polymorphisms upon the TB development. At the same time, several alleles of NRAMP1 were associated with severity of the disease, higher percent of segmental leukocytes and lower percent of monocytes (543N), higher percent of eosinophils (469+14C), lower level of hemoglobin and an increase of leukocytes (274T). Polymorphisms of VDR gene were associated mainly with roentgenological signs: both b and f alleles were associated with small size of cavities, whereas f alone was associated with small sizes of infiltrations, and b alone was associated with lung tissue destruction and decrease of stab leukocytes. A2+ allele of IL1RN gene was associated with lung tissue destruction and higher erythrocyte sedimentation rates, decrease of lymphocytes and thymol tests.

Thus, the studied genes NRAMP1, VDR, IL12B, IL1B and IL1RN in Tuvinians more likely influence the TB endophenotypes (acute phase reactions, size and degree of lung tissue destruction), but not the disease itself.

P1027. Distribution of cystic fibrosis mutations and the three microsatellite loci in Croatian population

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Cystic fibrosis is one of the most common recessive disorders in Caucasian affecting approximately 1 in 3 000 individuals. More than 1000 mutations have been identified in cystic fibrosis transmembrane conductance regulator gene (CFTR). The most frequent mutation, accounted for about 67% CF chromosomes, is ΔF508. Only four others (G542X, N1303K, G551D and W1282X) have frequencies higher than 1%; most other are rare and specific for some population subgroups. The southern-east region of Europe is highly heterogeneous and CF mutation analysis can be facilitated by association studies between intragenic polymorphic haplotypes.

The aim of this study was to reveal the frequency of 29 CF mutations along with the distribution of three polymorphic loci (IVS 1 CA, IVS 8 CA and IVS 17b CA) and associated haplotypes in diseased population. A total of 41 unrelated CF patients from Croatia were included in this study which revealed 6 different mutations accounted for 68,29% diseased alleles. The most frequent mutation was ΔF508 (58,74%), followed by G542X (3,66%) and N1303K (2,44%). Polymorphic loci analysis revealed high level of heterogeneity, 15 different haplotypes were found. The most frequent associated with CF was 21-23-13 (23,2%), followed by 21-17-13 (14,6%). ΔF508 mutation was associated with 5 different haplotypes, 21-23-13 being the most frequent one (31,3%). According to our results it can be concluded that genetic background of cystic fibrosis in Croatian population is more complex than it was expected. Much more work is needed to get inside the exact population screening with available kits and association studies.

P1028. Complex diseases: spurious evidence for a second gene when the effect of the first one is incorrectly specified

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Cordell and Clayton (*Am J Hum Genet*, 2002) proposed to use a stepwise logistic-regression procedure to evidence the effect of several variants in disease susceptibility. They applied this approach to test whether several genes of HLA are involved in the susceptibility to type

I diabetes. They concluded that DOB has an additional effect to the one of DRB1 in the disease susceptibility. In the above study, the DRB1 gene was considered as a biallelic susceptibility factor. However, such a model was strongly rejected by Clerget-Darpoux and Babron (*Genet Epidemiol*, 1989) who showed that at least a tri-allelic or more likely a complementation model could explain the HLA DRB1 observations in type I diabetes families. Consequently, we study the robustness of the stepwise logistic-regression in concluding to a second locus effect when the effect of the first one is incorrectly specified.

We simulate, in trio families (one patient and his two parents), a tri-allelic disease gene and another gene not involved in the disease but with linkage disequilibrium between alleles of the two genes. We apply the stepwise logistic-regression to these simulated data coding the disease gene either as tri-allelic or as bi-allelic.

When the disease gene is correctly coded as tri-allelic, we do not conclude to an additional effect of the other gene. In contrast, coding the disease gene as bi-allelic leads to falsely conclude to the involvement of the second gene. This emphasizes the importance of a correct modelling in the interpretation of a logistic regression analysis.

P1029. R121W polymorphism of PAX4 gene and risk of type I and type II diabetes in Russian North -West population.

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PAX4 gene plays a crucial role in the differentiation of insulin-producing β -cells in the pancreatic islets of Langerhans. The R121W mutation in the exon 3 of PAX4 has been reported to be a possible susceptibility polymorphism to the Type II diabetes in Japanese, while the investigation of this and other polymorphisms has shown no evidence of linkage with onset of Type II Diabetes in French population and in Ashkenazi Jews.

In this study we examined the association of R121W mutation with Type I and Type II Diabetes in Russian North-West population.

We investigated 121 children (62 girls, 59 boys) aged 3 to 17 with Type I Diabetes and 36 grown-ups (17 women, 19 men) aged 46 to 95 with Type II Diabetes, complicated with the neuropathy of the lower limbs. The control group included 142 healthy children. Identification of R121W PAX4 gene polymorphism was carried out by means of polymerase chain reaction followed by restriction.(Shimajiri et. al., 2001).

We didn't find mutant alleles in Type I and Type II Diabetes no in control group.

Thereby, the explored population seems to be homogeneous on R121W polymorphism and this mutation has no evidence of linkage with either Type I or Type II Diabetes in the Russian North-West population.

P1030. Interim CARD15 genotype results from a population-based study of Inflammatory Bowel Disease

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Three single nucleotide mutations (R702W, G908R & 1007fs) within the CARD15 gene are well recognized risk factors for the development of Crohn's disease (CD) in Caucasians. However, what has not been clearly established is whether these mutations can also be used to predict clinical parameters of CD. To date, almost all studies examining possible links between CARD15 genotype and clinical course of CD have used referral-based patient samples. These studies inherently suffer selection bias as only extreme cases of CD are included. We sought to clarify the potential predictive value of CARD15 genotype by examining all cases of inflammatory bowel disease (IBD) within a geographically defined region of New Zealand. It is hoped that this population-based approach will eliminate the selection bias seen in many previous studies. Since recruitment was initiated in 2003, DNA samples, epidemiological data and clinical history have been collected from 965 IBD patients (out of a total predicted population of 1400). CARD15 genotype has been established for 922 of these IBD patients and 200 controls. Interim analysis of CARD15 genotype data has found a significant increase in the frequency of individuals with two

CARD15 mutations in the IBD population compared to controls. Unlike previous studies, the elevated frequency of CARD15 mutations was not restricted to CD patients but also observed in patients with ulcerative colitis. Once recruitment and genotyping is complete it is anticipated that this population-based study will provide valuable insights into the role CARD15 mutations play in the development and progression of IBD.

P1031. Novel variants located within the intron 1 and the promoter region of the RET proto-oncogene are associated to the sporadic forms of Hirschsprung disease

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Several studies have demonstrated the existence of specific haplotypes of the RET proto-oncogene associated with the sporadic forms of Hirschsprung disease (HSCR). Based on such studies, Linkage Disequilibrium (LD) mapping estimates predicted the presence of a HSCR locus located at 22-50 kb upstream of codon 45 (exon 2) of RET.

Our aim was the identification of such a founding locus responsible for the majority of the HSCR cases of our series.

After a systematic mutational screening upstream of exon 2 of RET, we found a wide spectrum of new polymorphisms, all of them presenting with a distribution significantly different among cases and controls ($p<0.00002$). We identified a specific haplotype clearly linked to HSCR. Such LD was maintained along the whole region studied until position -1249. We noted that the ancestral haplotype associated with HSCR was characterised by the presence of specific SNP's (at -200 and -196) in proximity to the transcriptional start site. Functional modelling using luciferase expression assays revealed a significantly depressed activity for the HSCR-linked haplotype at -200/-196 in comparison with other combinations associated with controls.

Our results seem to discard the existence of an HSCR-causing mutation as it is conceived in the traditional sense, but strengthen the concept of a specific combination of markers conferring susceptibility to the disease in a low penetrance fashion. It would be conceivable that such HSCR-haplotype together with other events occurring in other genes, might give raise to the disease, which would be concordant with a polygenic model for the disease.

P1032. Statistical power of the HapMap study design

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The International Haplotype Map ('HapMap') Project aims to characterize genome-wide SNP-based gametic disequilibrium (GD) patterns to facilitate the discovery of genes that influence complex human diseases. Here we undertook an evaluation of the statistical power of the study design currently being used by The International HapMap Consortium for detecting different levels of GD between pairs of SNPs. The initial phase of HapMap is scheduled to genotype 270 individual samples from four widely distributed geographic regions for about 600,000 SNPs spaced at approximately 5-kilobase intervals and each with major allele frequency (MAF) < 0.95 . Sample sizes across populations are 45 and 90 individuals. Our observations show that the power of this study design is highly heterogeneous, and often fairly low under a variety of pairwise GD scenarios. Lack of power can lead to erroneous estimates of the size and number of blocks, as well as of the total number of haplotype-tagging SNPs (htSNPs) associated with block partitioning. In addition, heterogeneity of power can be a very relevant factor hindering comparisons of block structure both across genomic regions and among samples of varying sizes. Our analyses suggest that about 50- to 100-fold larger sample sizes than those currently used are required to effectively map genome-wide patterns of GD with estimation procedures used in HapMap.

P1033. Frequency of Interleukin-2 gene promoter polymorphism at position -330 among Iranian patients with chronic hepatitis B infection in Iran

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Background: The clinical course of hepatitis B virus (HBV) infection varies from spontaneous recovery to chronic persistent infection depending on viral and host factors. 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 (IL-2) and interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) have been identified as participating in the viral clearance and the host immune response to HBV. In this study we have evaluated IL-2 polymorphism in patient with chronic HBV infection.

Material and Method: We investigated -330 IL-2 polymorphism in 96 patients with chronic HBV infection, 96 subjects spontaneously recovered from acute HBV infection, and 95 healthy controls. After receiving an informed consent, genomic DNA was extracted from peripheral blood leukocytes by salting-out method. The -330 T/G polymorphism in the promoter region of IL-2 gene was detected by PCR-RFLP.

Results: The prevalence of -330 IL-2 polymorphism is shown in the table below, obtained by Chi-Square test in SPSS 11.5 software. The -330 G promoter allele frequency was 49.4%, 47.3%, and 47.8% in chronic, spontaneous recovered patients and controls, respectively.

Conclusion: These findings suggest no association between the IL-2 promoter polymorphism at position -330T/G and the development of chronic HBV infection ($p=0.83$).

The frequency of IL-2 (-330 T/G) polymorphism in studied groups			
	T/G or G/G	T/T	Total
Chronic	73 76%	23 24%	96 100%
Spontaneous Recovered	70 72.9%	26 27.1%	96 100%
Control	69 72.6%	26 27.4%	95 100%
Total	212 73.9%	75 26.1%	287 100%

P1034. Refined mapping of the autosomal recessive non-syndromic deafness locus DFNB13 using eight novel microsatellites markers

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The locus for a type of an autosomal recessive non-syndromic deafness (ARNSD), DFNB13, was previously mapped to 17-CM interval of chromosome 7q34-36. In order to refine this interval, we identified nine dinucleotide repeats in the 7q34 region. To investigate the polymorphism of these repeats, a population study of 74 unrelated individuals from different regions of Tunisia was carried out. Our results demonstrated that eight of the nine repeats are polymorphic. The average number of alleles at these informative loci was 9.12 with a polymorphism information content of 0.71. Little evidence for linkage disequilibrium between some markers pairs was found. Haplotype analysis using these markers refined the DFNB13 interval to an area of 2.2 Mb between the D7S5377 and D7S2473. In order to identify the DFNB13 gene, we sequenced and eliminated three candidate genes. Other known and predicted genes are being screened for deafness-causing mutations.

P1035. Short tandem repeats haplotyping of the HLA region in preimplantation HLA matching

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Recently, Preimplantation Genetic Diagnosis (PGD) has been considered for several indications beyond its original purpose, not only to test embryos for a genetic disease, but also to select embryos for a non-disease trait, such as specific Human Leukocyte Antigen (HLA) haplotypes, related to immune compatibility with an existing affected child in need of an hematopoietic stem cell transplant.

We have optimized an indirect single-cell HLA typing protocol based on a multiplex fluorescent polymerase chain reaction (PCR) of short tandem repeat (STR) markers scattered throughout the HLA complex. The assay was clinically applied in 60 cycles (54 for β -thalassemia, 1 for Wiscott-Aldrich syndrome, 2 for Diamond-Blackfan anemia and 3 for Acute Lymphoid Leukaemia) from 45 couples overall, involving the testing of 486 embryos in combination with a genetic disease and 44 embryos for HLA matching only. In 848/922 (92.0%) of blastomeres a successful amplification was achieved, obtaining a conclusive HLA matching diagnosis in 483/530 (91.1%) of the embryos tested. In total, 74 (15.3%) embryos revealed an HLA match with the affected siblings, 55 (11.4%) of which resulted unaffected and 46 (9.5%) have been transferred back to patients in 30 clinical cycles. Nine pregnancies were achieved, 5 healthy HLA matched children have been already delivered and cord blood stem cells were transplanted to 3 affected siblings, resulting in a successful hematopoietic reconstruction. Multiplex PCR of STR markers located in the HLA region has revealed a reliable diagnostic tool for indirect HLA matching evaluation in single cells.

P1036. Growth hormone (GH) receptor codon 437* T allele is related with GH therapeutic efficacy

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Objective: Growth hormone (GH) replacement is an effective therapy for the GH deficient (GHD) children. However, the height gain post GH replacement therapy varied differently between individuals. In this study, we aimed to investigate the correlations of the different gene polymorphisms and the GH efficacy.

Patients and methods: A total of 100 GHD children (73 male, 27 female) who accepted one-year GH treatment were included. All individuals were divided into three groups: (1) GHD; (2) 126 children with familial short stature (FSS, n=126) (3) normal controls (n=100). Gene polymorphisms for GH receptor (codon 437, 488, 492, 540), Janus kinase 1305, signal transducers and transactivators of transcription -5a codon 812, insulin-like growth factor I codon 230, IGF binding protein-3 codon 2605, and acid-labile subunit codon 1285 were detected by the PCR-RFLP (restriction fragment length polymorphism). The correlations of these gene polymorphisms and other parameters (sex, age, bone age, parents' heights, serum GH concentration, pituitary size, and birth weight) upon first year growth velocity were evaluated.

Results: The G homozygote, G/T heterozygote and T homozygote for GHR codon 437 appeared the lower, moderate, and height growth velocity. Other gene polymorphisms and parameters were non-correlated with the GH-therapy efficacy. The G-related genotype and allele were also related with higher susceptibility of FSS.

Conclusions: GHR codon 437* T allele is associated with therapeutic efficacy of GH replacement and lower susceptibility of FSS. GHR codon 437 gene polymorphism might become a useful marker for the pre-treatment evaluation of GHD children.

P1037. Screening for DFNB1, DFNB2, DFNB3, DFNB4, DFNB9 and DFNB21 loci in the Iranian patients with autosomal recessive non-syndromic hearing loss

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Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common form of severe inherited hearing impairment. To date, at least 43 loci for ARNSHL have been identified, indicating it as an extremely heterogeneous disorder. These loci are referred to as DFNB loci. Although, mutations at the DFNB1 locus (including GJB2 and GJB6 genes) are the most common cause of ARNSHL in populations originating from Northern Europe, we have shown that mutations in GJB2 and the Δ (GJB6-D13S1830) do not play a significant role in the etiology of deafness in Iran. In this study, we assess the contributions made by other loci to the ARNSHL genetic load in Iran. We have selected 50 consanguineous families with normal GJB2 and GJB6 alleles to be screened for linkage to the DFNB2, DFNB3, DFNB4, DFNB9 and DFNB21 loci. Linkage analysis is applied using, in average, 3 short tandem repeat (STR) markers for each locus. These loci have been excluded in 11 families but two families; each has been localized to DFNB4 and DFNB21. We are screening these families for mutations in SLC26A4 and TECTA, respectively. Our results suggest that other loci may have the major causative roles in ARNSHL in Iran. This hypothesis will be confirmed by including more families and screening additional loci.

P1038. Colobomatous macrophtalmia with microcornea syndrome maps to 2p23-p16 region containing CYP1B1, SOS1 and SIX2 and 3 genes

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P1039. Genetic Heterogeneity of Pelizaeus-Merzbacher Disease: Exclusion of Linkage to the Proteolipid Protein 1 Locus in Three Affected Families

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Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive hypomyelinating disorder of the central nervous system (CNS). The clinical severity in PMD varies, but the common characteristics include nystagmus, psychomotor retardation, spasticity and ataxia. PMD is primarily caused by mutations in the proteolipid protein 1 (*PLP1*) gene

on chromosome Xq22. About 20% of PMD cases have been shown to carry no mutation in the *PLP1* gene suggesting involvement of other loci or mutations in noncoding regions of the *PLP1* gene.

In the present study, we tested the genetic homogeneity of PMD by performing linkage analyses in three PMD families. A common haplotype was found in all affected individuals, their asymptomatic mothers, and in two other asymptomatic females in the first family. X chromosome inactivation (XCI) analysis revealed presence of the same active chromosome in both affected and unaffected individuals ruling out the *PLP1* region as the causative locus in this family. The two affected brothers in the second family and the two affected sisters in the third family, both born to unaffected parents, were found to inherit different maternal haplotypes for the *PLP1* locus. The lod score value was less than zero at $\theta = 0.00$ confirming the results of haplotype analysis.

The exclusion of linkage to the *PLP1* locus in these families suggests existence of at least one other locus and presence of genetic heterogeneity in PMD.

P1040. Genotype distribution and allele frequencies of the endothelial nitric oxide synthase gene polymorphism (G894T, 4a4b, T-786C) in women with coronary heart disease (CAD) in St. Petersburg, Russia.

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Endothelial dysfunction is a key step in both initiation and progression of atherosclerosis. Nitric oxide is synthesized from L-arginine by endothelial nitric oxide synthase encoded by eNOS gene. Mutations affecting the eNOS gene, and consequently impairing NO availability, might contribute to increased predisposition to CAD.

We designed this study to investigate the role of these 3 types of polymorphisms in the eNOS gene as predisposing factors to CAD in women.

In 79 angiographically diagnosed CAD women and in 145 unrelated controls genotypes of eNOS G894T and T-786C polymorphism were determined by polymerase chain reaction-restriction fragment length polymorphism analysis and the repeat polymorphism 4a4b was analyzed by polymerase chain reaction. The genotype distribution was in Hardy-Weinberg equilibrium for all variants. Statistical significance of differences between groups was assessed with χ^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 eNOS gene and the presence of vascular disease states.

Genotype	Allele	Controls n (%)	Patients n (%)	P-value
4b4b		90 (62.9)	56 (70.9)	0.232
4a4b		45 (31.5)8 (5.6)	20 (25.3)3 (3.8)	
4a4a				
894GG	4a	61 (21.3)	26 (16.5)	0.216
		77 (52.4)	37 (49.3)	
		58 (39.5)	29 (38.7)9 (12.0)	
894TT		12 (8.1)		0.783
-786 TT		82 (27.9)	47 (31.3)	0.548
		56 (38.4)	32 (40.5)	
		70 (47.9)20 (13.7)	39 (49.4)8 (10.1)	
-786 C-786 CC				0.647
-786 CC		110 (37.7)	55 (34.8)	0.450

P1041. Classification of haplotypes based on evolutionary relationships

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Increasing numbers of marker loci make the number of different haplotypes too large, which reduces statistical power to an insufficient

level to detect an association between single haplotype and disease. To obtain the sufficient statistical power, classification of haplotypes is often carried out. Many of the methods of the classification, including hierarchical classification and partitions, require measures of similarity. Appropriate choice of the measure is a key factor to obtain good classification. However, the usual measures using the numbers of different variant sites do not always reflect the relationships of haplotypes. In the present study, we have defined a new measure based on evolutionary relationships between two haplotypes, and applied the measure to the classification of haplotypes.

The presented approach is performed as follows. We first build the maximum likelihood evolutionary tree from observed haplotypes, by assuming that each edge represents a single evolutionary change (i.e. single mutation or recombination). To satisfy this assumption, some unobserved haplotypes are incorporated as nodes to complete the tree if required. Once the tree is built, the maximum likelihood path between two haplotypes is computed from probabilities of mutation and recombination. We define the likelihood as the measure of similarity. We then carry out the haplotype classification, by both common hierarchical classification and partitions, using the defined measure. We have validated our approach using synthetically created datasets. The datasets are created by mixing haplotypes derived from different ancestors. The resultant classification shows good agreement with the given ancestors.

P1042. Association of genetic polymorphisms in the ACE, BCHE and APOE genes with Late-Onset Alzheimer Disease in the Korean population

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Apolipoprotein E(APOE) ε4 allele and Angiotensin Converting Enzyme deletion(ACE-D) have been reported to have an association with Late-Onset Alzheimer Disease(LOAD). Butyrylcholinesterase(BCHE) is also known to be up-regulated in the Alzheimer Disease(AD) brain, and the increased odds of carrying the K variant of BCHE(BCHE-K) was indicated among AD cases as compared with controls. The objective was to determine if these DNA polymorphisms at the ACE, BCHE and APOE genes that have been linked with different levels of enzyme expression, have some effect on the risk of developing LOAD. In order to verify the association of ACE and BCHE genes with AD, as well as its association with APOE genotype, ACE and BCHE genotyping were performed in subjects with 62 LOAD cases and 190 healthy controls including 68 healthy age-matched controls, who were previously characterized for APOE gene. After component analyses, ACE-D, BCHE-K and APOE ε4 alleles disclosed the highest prevalence in the AD group ($P<0.05$). A comparison between the AD and the healthy individuals, both with the APOE ε4 allele, indicated an interaction between the BCHE-K and the APOE ε4 allele($p<0.05$). The association of the BCHE-K with AD was limited to carriers of the APOE ε4 allele, among whom the presence of the BCHE-K gave an odds ratio of AD 3.48 (95% C.I. 1.3-9.2). Therefore, ACE D allele seems to be a susceptibility factors for the AD with the APOE ε4 allele, and the BCHE-K variant acts in synergy with the APOE ε4 allele as a susceptibility genes for AD.

P1043. Genetic association of vitamin D receptor polymorphisms with autoimmune hepatitis in Iranian patients

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Autoimmune hepatitis (AIH) is an immune mediated disorder of the liver with unknown etiology. 1, 25 dihydroxyvitamin

D₃ has immunomodulatory capabilities which extends its biological effects through the vitamin D receptor (VDR). The aim of this study was to investigate the association of VDR polymorphisms in Iranian patients with AIH. METHODS: In this study we investigated the frequency of the VDR polymorphisms in a case-control study between 71 patients with AIH (53 female, 18 male), referred to Taleghani hospital (Iran), and 109 healthy blood donors (73 female, 36 male) matched by sex and age after obtaining the informed consent. The mean age for

cases and controls were 36.6 ± 13.5 and 35.0 ± 12 , respectively. After DNA extraction by salting out method, polymorphisms of FokI, BsmI, Apal and TaqI were detected by PCR RFLP method. RESULTS: The VDR genotype distribution in patients and controls are shown in Table 1. The frequency of A, B, F, and T alleles are 60.5% vs. 58.7%, 47.1% vs. 38.9%, 74.6% vs. 75.2%, 63.3% vs. 71.1% in cases and controls, respectively. Analysis of allelic frequencies revealed no significant difference between AIH patients and controls for either of the polymorphisms ($P>0.05$). CONCLUSION: Our results do not confirm the association between VDR polymorphisms and AIH. As a consequence, these polymorphisms cannot possibly be considered as biomarkers, at least, in Iranian patients with autoimmune hepatitis.

	Case		Control	
	Female (%)	Male (%)	Female (%)	Male (%)
AA	16 (30)	7 (39)	25 (34)	11 (31)
Aa	31 (58)	9 (50)	38 (52)	18 (50)
aa	6 (11)	2 (11)	10 (14)	7 (19)
BB	9 (17)	5 (28)	14 (19)	3 (8)
Bb	29 (55)	10 (55)	31 (42)	20 (55)
bb	15 (28)	3 (17)	28 (38)	13 (36)
FF	34 (64)	6 (33)	39 (54)	23 (64)
Ff	16 (30)	10 (56)	27 (36)	13 (36)
ff	3 (6)	2 (11)	7 (9)	0 (0)
TT	23 (43)	5 (28)	35 (48)	16 (44)
Tt	22 (42)	12 (67)	34 (47)	19 (53)
tt	8 (15)	1 (5)	4 (5)	1 (3)

P1044. DNA typing in Tatar patients with type 1 diabetes mellitus

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Type 1 diabetes mellitus (T1DM) is caused by interplay of genetic and environmental factors. Human leukocyte antigen system (HLA) has been demonstrated to contribute to T1DM genetic susceptibility. Tumor necrosis factor α and interleukin 1 β activate inducible NO-synthase in β -cells causing their destruction. So, the genes, encoding these cytokines, can be supposed as candidate genes for T1DM. We aimed to analyze the relationship between HLA class II DRB1 and DQB1 genes, G-308A polymorphism of tumor necrosis factor α gene (TNFA) and T-511C polymorphism of interleukin 1 β gene (IL1B) and susceptibility to T1DM in Tatars of Bashkortostan. We PRC-analyzed DNA of 111 T1DM Tatar patients and 232 Tatar controls. The HLA-DRB1 (investigated in 87 T1DM patients and 97 controls) and HLA-DQB1 (analyzed in 46 T1DM patients and 22 controls) genomic typing revealed increased frequencies of DRB1*04 (27.6% vs. 9.3%, $P<0.001$), DRB1*08 (8.6% vs. 1.0%, $P<0.001$), DQB1*02 (33.7% vs. 15.9%, $P=0.041$), DQB1*0302/08 (26.1% vs. 6.8%, $P<0.01$) in T1DM patients compared to controls. The frequencies of genotype AG (32.4% vs. 19.4%, $P<0.01$) and allele A (19.8% vs. 10.6%, $P=0.001$) of the TNFA G-308A polymorphism were higher in T1DM patients ($n=111$) than in controls ($n=232$). Concerning T-511C polymorphism of IL1B gene, we couldn't find differences of the genotype and allele frequencies between T1DM patients ($n=60$) and controls ($n=166$). Thus, the genetic markers of T1DM in Tatars from Bashkortostan are DRB1*04, DRB1*08, DQB1*02, DQB1*0302/08 of HLA class II DRB1 and DQB1 genes, genotype AG and allele A of G-308A polymorphism of TNFA gene.

P1045. No association of the polymorphisms (TTTTA)_n in the CYP11A gene and VNTR in the INS gene with polycystic ovary syndrome

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Hyperandrogenism and insulin resistance are cardinal features of polycystic ovary syndrome (PCOS). Among several PCOS candidate genes, the gene encoding P450scc (CYP11A gene) and the insulin (INS) gene have been proposed.

The aim of the study was to investigate an association of the (TTTTA)_n microsatellite polymorphism in the CYP11A gene and the minisatellite polymorphism (VNTR) in the INS gene with PCOS.

Forty-six PCOS women and 110 control patients were genotyped for the genetic polymorphisms in the *CYP11A* and *INS* gene.

The *CYP11A* allele frequencies were 0.56, 0.27, 0.09, 0.09 in the study and 0.55, 0.28, 0.09, 0.09 in the control group, for alleles with four, six, eight and nine TTTTA repeats, respectively. Eighty-five percent of PCOS patients vs. 80.9 % controls had 4+ genotype (with at least one copy of the four-repeat-unit allele), and 15.0 % patients vs. 19.1% controls had 4- genotype (without the four-repeat-unit allele).

In the *INS* gene, allele frequencies for class I and class III alleles were 0.25 and 0.75 in the study vs. 0.30 and 0.70 in the control group. The genotype frequencies were 0.54, 0.41 and 0.05 in the study vs. 0.49, 0.42 and 0.09 in the control group, for genotypes III/III, I/III and I/I, respectively.

None of the alleles or genotypes in the two genes was statistically differently distributed between the study and the control group of patients.

Our results suggest that the *CYP11A* (TTTTA)_n and the *INS* VNTR allelic variants are not likely to be associated with PCOS in Slovene patients.

P1046. Is p.S1235R a cystic fibrosis causing mutation? Results from a French molecular collaborative study

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More than 1300 different mutations in the CFTR gene, causing or non-causing disease, have been reported to the CF Consortium. p.S1235R, initially reported in a CF patient with a second mutation on the same allele, has been found at a frequency higher than that of many other mutations and its clinical significance is not clear.

The aim of this study is to compare the phenotypes of 57 subjects with p.S1235R in order to classify this sequence anomaly. They were referred for diagnosis of classical CF, non-classic or atypical phenotypes or carrier screening. The entire coding and flanking regions and six microsatellite markers were analyzed. 8 patients (3 CF, 4 CBAVD, 1 ICP) and 2 normal individuals were compound heterozygotes for a severe CFTR mutation. p.S1235R was found to be associated on the same allele with a stop mutation (p.R785X) in 2 CF patients with a severe disease and TG13-T5 in the 4 CBAVD patients. The CF patient with a mild phenotype, the ICP patient and the normal subjects did not carry a complex allele.

Our data suggest that another CFTR mutation may influence the pathogenic effect of p.S1235R in severe classical CF or in patients with CFTR-opathies. However, p.S1235R alone, when combined in trans with a second CF mutation, may be associated with a mild phenotype or with absence of clinical manifestations.

P1047. Association analysis of neurotransmitter systems of brain genes with paranoid schizophrenia in different ethnic origin groups from Russia

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Schizophrenia is a chronic debilitating psychotic mental disorder that affects about 1 percent of people.

The aim of our study was to test a contribution of 9 polymorphisms of some candidate genes in paranoid schizophrenia, namely -5-HTTLPR and VNTR in the serotonin transporter (*SLC6A4*), *MspI* in the serotonin 2A receptor (*HTR2A*), VNTR in the dopamine transporter (*SLC6A3*), *TaqI*A and *Ncol* in the dopamine D2 receptor (*DRD2*), A/G polymorphism in intron 13 in the monoamine oxidase A (*MAO A*), *Hsp92II* in the catechol-O-methyltransferase (*COMT*) and *NlaIV* in the brain - expressed protein (*G72*) genes.

351 patients (131 Russians, 112 Tatars and 108 Bashkirs) with paranoid schizophrenia (diagnosed as having ICD-10) at the age of

15 - 74 and 423 control subjects (115 Russians, 168 Tatars and 140 Bashkirs) were genotyped using PCR- technique and subsequent enzyme digestion.

We found a significant association of the *COMT**H/*H genotype with schizophrenia in different ethnic origins groups (Tatars: OR=2.38, 95%CI=1.19-5.64; Russians: OR=2.83, 95%CI=0.89-4.86; Bashkirs: OR=2.11, 95%CI=1.22-6.23).

It was shown associations with schizophrenia of the *SLC6A4* *S/*S genotype (OR=2.04, 95%CI=0.98-4.27), of the *HTR2A* *A/*A genotype (OR=2.21, 95%CI=0.97-5.04) and of the *DRD2* *N1/*N1 genotype (OR = 2.8, 95% CI=1.33-5.92) in Tatars; of the *DRD2* *A1/*A2 genotype (OR=2.13, CI=1.02-4.47) in Bashkirs; of the *SLC6A3* *9/*9 genotype (OR=17.28, 95%CI=2.09-3.99) in Russians.

The obtained results are interesting for greater understanding of molecular-genetic mechanisms of predisposition to schizophrenia.

P1048. The role of *GSTM1*, *CYP1A1*, *CYP2D6*, *APOE* gene polymorphisms in sporadic Parkinson's disease development

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Parkinson's disease (PD) is a complex disorder with multiple genetic and environmental factors. The majority of PD cases (80-90%) are sporadic. Several causal genes for familial PD have been identified, but genetic factors associated with sporadic PD haven't been ascertained. Genes involved in the process of xenobiotic detoxication can play a causal role in PD development.

We've performed analysis of some gene mutations and polymorphisms in 200 sporadic PD patients and 210 controls to investigate their role in disease development.

Analysis of *GSTM1* gene revealed higher frequency of *GSTM1*-deficient genotype in PD patients in comparison with controls that determines low level of enzyme activities in patients. Differences were significant ($\chi^2 = 8.21, P=0.005$, OR=1.45, CI=1.20-2.83). These data confirm the hypothesis that *GSTM1* gene, coding antioxidative enzyme and playing a major role in toxins elimination, may be involved in PD pathogenesis. *CYP1A1* and *CYP2D6* polymorphisms didn't show significant differences between patients and controls.

One of genetic neurodegeneration markers is *APOE* gene polymorphism. It is considered that *E4 allele plays a modifying role in some neurodegenerative diseases, but its role in PD development remains contradictory. We found significant differences in allele and genotype distribution between patients and controls ($\chi^2 = 10.43, P=0.005$). Analysis of *E4 allele distribution showed significant differences between PD patients of Tatar origin and control group of Tatars ($\chi^2 = 7.45, P=0.007$, OR=4.12), PD patients of Russian origin and control group of Russians ($\chi^2 = 5.60, P=0.01$, OR=4.82). So, allele *E4 is a risk factor of PD development in Tatars and Russians in the Volga-Ural region.

P1049. Association of VDR Bsm I Polymorphism with Prostate Cancer in Turkish Population

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OBJECTIVE: To investigate the association between VDR gene Bsm I polymorphism and prostate cancer in Turkish population.

METHOD: DNA was extracted from whole blood of 39 prostate cancer cases and 52 controls by Qiagen DNA extraction kit. The area of which Bsm I polymorphism located in 8th intron of VDR gene was amplified by PCR. Subsequently, PCR products have been digested by Bsm I restriction enzyme and results were analysed by agarose gel electrophoresis.

RESULTS: Bsm I BB, Bb and bb genotype frequencies are not significantly different between cases and controls ($P>0.05$). Bsm I Bb genotype frequencies were 56.4 % and 57.6 %, BB genotype frequencies were 20.5 % and 26.9 %, bb genotype frequencies were 23% and 15.3 % in cases and controls respectively.

CONCLUSION: Our findings did not confirm an association between VDR gene Bsm I polymorphism and prostate cancer in Turkish population.

P1050. Association of VDR Gene Apa I Polymorphism with Bone Mineral Density in Obese Postmenopausal Women

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To investigate the incidence of Vitamin D receptor gene polymorphism and look for the association between VDR gene polymorphism Apa I genotypes and BMD of obese postmenopausal women in Turkish population. Genomic DNA of 87 nonosteoporotic and 72 osteoporotic subjects was extracted from whole blood. In order to detect the Apa I polymorphism in the 8th intron, genomic DNA was amplified by PCR and PCR products were further digested by Apa I restriction enzyme. The fragments were detected by agarose gel electrophoresis. Comparison of genotype frequencies of osteoporotic and nonosteoporotic obese postmenopausal women was done by using Chi square method. Differences in bone density at different skeletal sites (BMD1=Lumbar Spine) and (BMD2= Femoral Neck) between the osteoporotic and nonosteoporotic obese postmenopausal women defined by VDR genotypes were determined by using variance analysis and BMD values of subjects with different genotypes were compared by t-test. It was observed that the frequency of aa genotype in osteoporotic obese postmenopausal women were increased compared to nonosteoporotic subjects. When we compared BMD-VDR gene polymorphism genotype relationship within the group, nonosteoporotic obese Aa postmenopausal women had $0.98 \pm 0.094 \text{ g/cm}^2$ lumbar spine BMD and it was higher compared to AA subjects who had $0.92 \pm 0.060 \text{ g/cm}^2$. No significant differences were detected between the genotypes and lumbar spine BMD in the osteoporotic obese group. However aa subjects had the lowest femoral neck ($0.67 \pm 0.082 \text{ g/cm}^2$).

P1051. Evaluation of RET variants and haplotypes as susceptibility factors for sporadic medullary thyroid cancer

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Medullary thyroid carcinoma (MTC) is a neoplasm of the C cells of the thyroid comprising about 5-10% of all thyroid malignancies. About 25% of all MTCs are believed to be inherited and grouped in a cancer syndrome known as Multiple Endocrine Neoplasia type 2 (MEN 2), while the remaining 75% of all MTCs are sporadic (sMTC). While germline gain-of-function mutations in the *RET* proto-oncogene cause hereditary MTC, the molecular mechanisms leading to the sporadic forms remain obscure.

Our group had evidence about the existence of a low-penetrance susceptibility locus for sMTC in linkage disequilibrium with *RET* variants S836S and IVS1-126G>T, and probably in 5' with respect both variants. To identify such locus, we performed a case-control study analysing a wide spectrum of *RET* variants in the 5' region of the gene. On the other hand, since an over-representation of G691S/S904S variants in sMTC patients had been previously reported by other groups, we sought to determine if such association was also present in our series.

No differences were obtained among cases and controls in the distribution of all the variants tested. The whole findings would suggest that the major genetic events contributing to the appearance of sMTC may reside in several different *RET* loci. In this way, we could hypothesise about the existence of at least two sMTC loci, linked to S836S-IVS1-126G>T, or to G691S-S904S respectively. The characterization of those loci is a challenge, and would have important repercussions in the management of medullary thyroid carcinoma.

P1052. Association of VDR Gene Taq I Polymorphism with Bone Mineral Density in Obese Postmenopausal Women

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The relationship between VDR gene Taq I genotypes and BMD of obese postmenopausal women in a Turkish population was addressed in this study. Following the extraction of genomic DNA of 87 nonosteoporotic and 72 osteoporotic subjects, Taq I polymorphism was analysed in the 9th exon of the gene. The genotypes and their relations with the clinical features [bone density at different skeletal

sites (BMD1=Lumbar Spine) and (BMD2=Femoral Neck)] of the cases were compared. Tt heterozygote subjects had the highest lumbar spine BMD ($0.98 \pm 0.099 \text{ g/cm}^2$) compared to TT and tt subjects, whereas no significant differences were seen between the genotypes and lumbar spine BMD. However, tt subjects had the highest femoral neck BMD ($0.81 \pm 0.05 \text{ g/cm}^2$).

P1053. Association of genetic polymorphisms in ERα, ERβ and AR genes with osteoarthritis

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To elucidate the possible role of genetic variation in the estrogen receptor α (ER-α), β (ER-β) and androgen receptor (AR) genes with osteoarthritis of the knee, the (TA)n, (CA)n and (CAG)n repeat polymorphisms of ER-α, ER-β and AR genes were studied.

A case-control cohort of 94 patients with idiopathic knee osteoarthritis who had undergone total knee replacement (TKR) and 123 unaffected controls were used. The (TA)n, (CA)n and (CAG)n repeat polymorphisms of the human ER-α, ER-β and AR genes were analyzed using an automated DNA analysis method.

An association was observed between (CA)n and (CAG)n repeat polymorphisms in ER-β and AR genes and knee OA ($p=0.000$ and $p=0.007$), while no association was found between (TA)n repeat polymorphism in the ER-α gene and knee osteoarthritis ($p=0.3$). The mean number of (CA)n repeats was higher in OA individuals than controls, while the mean number of (CAG)n repeats was lower in men with OA compared to controls. This was reflected in a significantly increased odds ratio (3.5 and 3.6 respectively) of knee OA in individuals possessing alleles with more than 21 CA repeats, and having LL or SL genotypes, (95% CI 1.7-7.5, $p=0.001$) compared to individuals with the SS genotype, while men having the L genotype of CAG repeats ($OR=0.3$) had decreased odds ratio for knee OA compared to those with S genotype (95% CI 0.9-16, $p=0.063$).

An association between (CA)n and (CAG)n repeat polymorphisms in the ER-β and AR genes and knee osteoarthritis was found in individuals of Greek descent.

P1054. Prevalence of pathogenic mutations in an Italian clinical series of patients with familial dementia

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Mutations in the genes encoding for presenilin 1 and 2 (PSEN1, PSEN2), amyloid precursor protein (APP), and tau (MAPT) are associated with familial forms of dementia. Mutations in PSEN1 account for more than 50% of Alzheimer Disease cases with positive family history (FAD), while mutations in PSEN2 are more rare. Familial cases with an autosomal dominant inheritance have been estimated to have a frequency of APP mutations around 10-15%. Thirty to 50% of Familial Frontotemporal dementia (FFT) cases are explained by MAPT mutations.

Our aim was to investigate the prevalence of pathogenic mutations in an Italian clinical series of patients with a positive family history for dementia: we analyzed 13 patients with FAD and 40 patients with FFT. In the FAD group, we found two novel presenilin 2 mutations (M239I, T122R) and one previously reported mutation in presenilin 1 (P117L). No mutations were detected in APP gene. In the FFT patients group that we analyzed, we found just a patient bearing any mutation in the MAPT gene (tau-P301L). The communication of the presence of mutations occurred during genetic counselling sessions. The frequencies of mutations in PSEN1, PSEN2 and APP genes were 7.14%, 14.28% and 0% and, restricting the analysis to early onset FAD cases, the frequencies were 14.28%, 28.57% and 0%, respectively. Our data suggest that other gene mutations or additional genetic factors might be responsible for disease onset in most cases of dementia with a positive family history. The non-homogeneous distribution of pathogenic mutations might depend on a genetic drift.

P1055. Mutation in Lysosomal Acid Phosphatase (Acp2) causes cerebellum malformation by disrupting the synaptic transport

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Lysosomal acid phosphatase (Acp2) is a phosphoesterase of the endosomal-lysosomal compartment. Its expression in brain is considerably higher as compared to basal level ubiquitous expression. We have identified and characterized a mouse mutant named nax, which exhibit complete impairment of the cerebellar cytoarchitecture. In nax cerebellum the precursor external granule cells fails to migrate to form inner granular layer, Purkinje cell layer is disorganized, and Bergmann fibre structure is disrupted. Ultrastructural analysis of nax cerebellum showed lysosomal storage bodies in cerebellar cells. By linkage analysis and positional cloning, we identified a missense mutation (G244E) in Acp2 gene in nax mice DNA. Identification of Acp2 as the gene mutated in nax mice provides a valuable model system to study the role of Acp2 in cerebellum homeostasis and lysosomal storage disorder.

To decipher the underlying mechanism leading to cerebellum malformation currently our studies are focused on differential gene regulation in P5 stage nax cerebellum through microarray and real time RT-PCR analysis. We have identified 79 genes, which are either up-regulated or down-regulated in P5 stage of nax cerebellum as compared to normal wild-type siblings. Our initial analysis suggests that genes involved in synaptic vesicles and its transport are affected in nax mice cerebellum. Further analysis on this differentially regulated gene is in progress.

P1056. Polymorphism of the ACE(I/D), AGT (T174M), NOS1 (C/T) and NOS3 (VNTR, C691T, C774T, G894T) genes in patients with insulin-dependent diabetes mellitus

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Polymorphisms of NO-synthases and renin-angiotensin-aldosteron system genes could be one of the earliest marker of vascular damages in diabetes, when exacerbated hormonal and metabolic changes accompanying with activation of oxidative processes and infringement of the nitric oxide cycle exist.

An analysis of I/D polymorphism in the ACE gene and AGT (T174M), NOS1 (C/T) and NOS3 (VNTR, C691T, C774T, G894T) genes in patients with insulin-dependent diabetes mellitus (IDDM, n=113, 170 families) was carried out. 122 men without clinical symptoms of cardiovascular diseases and diabetes were studied as control.

There was an excess of the allele B of NOS3 gene (VNTR polymorphism) patients with diabetic nephropathies ($\chi^2=4.189$, $p=0.041$) and excess of the allele A in patients with ketoacidic conditions ($\chi^2=5.304$, $p=0.021$). An association between the polymorphism and IDDM was revealed: relative risk (RR) for NOS1 locus was 1.600 ($p=0.007$); RR for NOS3 C691T was 1.990 ($p=0.010$); RR for NOS3 C774T was 2.230 ($p=0.007$). Also, significant contribution of I/D polymorphism of ACE gene in a variability of some parameters of lipids metabolism, body mass index and insulin dose was found out. Using Transmission/Disequilibrium Test (TDT), we found an association between the NOS3 774C allele and IDDM (TDT=4.2, $p=0.016$). Finally, the 174T allele of AGT gene and the D allele of ACE gene were associated with diabetic retinopathies (TDT=3.765, $p=0.048$; TDT=8.522, $p=0.004$, respectively).

Thus, NOS-genes and renin-angiotensin-aldosteron system genes are the risk factors for IDDM and the disease-associated traits.

P1057. Identifying candidate Hirschsprung disease associated RET variants.

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Abstract Patients with sporadic Hirschsprung disease (HSCR) show an increased allele sharing at markers in the 5' region of the RET locus, indicating the presence of a common ancestral RET mutation. In a previous study, we found a haplotype of six SNPs that was transmitted to 55.6% of our patients, whereas it was present in only 16.2% of the controls we used. Among the patients with that haplotype, 90.8% had it on both chromosomes, which gave a much higher increased risk of developing HSCR than when the haplotype occurred heterozygously. In order to more precisely define the HSCR-associated region and to identify candidate disease-associated variant(s), we sequenced the shared common haplotype region from 10 kb upstream the RET gene through intron 1 (in total 33 kb) in a patient homozygous for the common risk haplotype and in a control individual homozygous for the most common non-risk haplotype. A comparison of these sequences revealed eighty-four sequence differences. Eight of these eighty-four variations proved to be in regions highly conserved among different vertebrates and within putative transcription factor binding sites. We therefore, considered these as candidate disease-associated variants. Subsequent genotyping of these eight variants revealed a strong disease association for six of the eight markers. These six markers also showed the largest distortions in allele transmission. Interspecies comparison showed that only one of the six variations was located in a region also conserved in a non-mammalian species, making it the most likely candidate HSCR associated variation.

P1058. Association analysis of serotonin transporter promotor region polymorphism 5-HTTLPR with personality risk factors associated with pathological gambling in Han Chinese population

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Family and twin studies have demonstrated evidence for genetic influence in the etiology of pathological gambling (PG). Recent molecular genetic studies suggest possible association of serotonin transporter gene (5-HTTLPR) with PG. At the same time, researchers have attempted to identify core personality trait of PGs. It is believed that people having certain personality traits maybe more vulnerable to the development of PG and the strongest evidence exists for impulsivity. However, the manifestation of PG should not be simply induced by either genetic or personality risk factors, but more likely the interplay between the two. This association study aims to examine the interrelationship between PG related personality risk factors and 5-HTTLPR in Han Chinese male.

134 PGs and 104 normal controls with mean age 41.84 and 40.80 years ($p=0.324$) were genotyped for 5-HTTLPR. Personality risk factors were identified through Zuckerman's Sensation Seeking Scale, Barratt's Impulsivity Scale and Chinese Personality Assessment Inventory. PGs as compared to controls have significantly higher sensation seeking, impulsivity, emotionality, external locus of control, pathological dependence and antisocial scores. No significant differences were observed in genotype ($p=0.963$) and allele frequency ($p=0.794$) of 5-HTTLPR between the two groups. Also, no association was found between 5-HTTLPR genotype and any of the personality risk factors identified. Since majority of gambling studies were based on Western samples, this negative finding for 5-HTTLPR as risk factor for PG may be attributed to ethnic difference or our modest sample size. However, the core personality traits identified can be good endophenotypes for further genetic studies.

P1059. Frequencies of HFE mutations in Russia

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Hereditary hemochromatosis (HH) is autosomal-recessive iron overload characterized by high polymorphism which embarrasses diagnostics. Estimation of the HFE main mutations' (C282Y and H63D) frequencies among HH and non-HH patients is necessary to determine role of the mutations and possibility to use them for diagnostics. We selected 54 patients matching clinical and laboratory criteria of HH.

Average age was 44.3 years (SD=13.3). Male/female ratio was 3.5:1. The second group (n=567) was formed from patients of the following departments: gastroenterology, hepatopathology, cardiology, endocrinology (diabetes). Average age was 50.1 years (SD=26.8). Male/female ratio was 1:1.2. Only 39 blood samples from 54 HH patients were available for genotyping: CC - 87.2%; CY - 7.7%; YY - 5.1%; HH - 51.3%; HD - 46.1%; DD - 2.6%. The second group was genotyped: CC - 94.5%; CY - 5.1%; YY - 0.4%; HH - 70.9%; HD - 26.6%; DD - 2.5%. C282Y frequency is surprisingly low among HH patients compared to European data. Controversy H63D is relatively frequent among HH patients. Allele frequencies in the second group does not differ from most population data in Russia and in Europe as well. However, H63D frequency is slightly higher in Russians. Though pathogenic genotypes (YY, DD, CY/HD) are quite frequent, clinically overt HH is very rare. Our data supports low penetrance of C282Y mutation and demonstrates also that it is necessary to search for new loci responsible for HH in Russia. Population screening of HH is not reasonable and use of HFE-genotyping should be restricted to very rare cases.

P1060. Association analysis of the candidate genes RELN, LAMB1, CUTL1 and NRCAM for autism on chromosome 7q

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Autism is a severe neurodevelopmental disorder that usually occurs due to a complex genetic predisposition. It is characterized by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities. Previously linkage for autism has been found to chromosome 7q, which has been replicated by many groups. Four candidate genes present in chr7q22-31 have been screened for mutations and association with autism in the IGSAC sample; *RELN*, *LAMB1*, *CUTL1* and *NRCAM*. Several missense mutations were found in *RELN* and new coding variants in *CUTL1* and *LAMB1*. Evidence for association was found with *LAMB1* and *NRCAM*.

To further analyse these genes an additional 31 SNPs were genotyped in 239 Affected Sibling Pairs from the IGSAC sample. Association analysis was carried out using the Transmission Disequilibrium Test (TDT) and some evidence for association was found with *LAMB1* ($p<0.05$). TRANSMIT was also run to test for haplotype transmission but no significant results were obtained. 192 controls were genotyped and their allele frequencies compared with unrelated affected individuals in a case-control analysis, which was carried out using Fishers exact test. A significant result was found for a SNP in *CUTL1* ($p<0.001$).

Although a denser marker map was used in this study to cover the candidate genes in question it was not exhaustive. To definitively exclude these genes further genotyping of an even greater number of markers would be required.

P1061. The influence of endothelin gene polymorphisms on the progression of autosomal dominant polycystic kidney disease (adpkd)

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A significant phenotypical variability is observed in ADPKD. Endothelin-1 (ET-1) is a promoting factor in renal diseases. We examined the influence of single-nucleotide polymorphisms of EDN1 - K198N, 3A/4A and T-1370G - on the progression of ADPKD.

205 ADPKD patients (pts) were analyzed. Pts were divided in three groups: 1. 48 pts with ESRF later than in 63 years (slow progressors), 2. 83 pts with ESRD between 45-63 years and 3. 74 pts with ESRF before 45 years (rapid progressors). DNA samples were genotyped for three single-nucleotide polymorphisms of EDN1. The EDN1 genotype distribution showed no differences between slow progressors and rapid progressors. We did not find significant differences in the ages of end stage renal disease (ESRD): 1. K198N - KK (51.9±8.8 years), KN (50.4±8.6 years), NN (51.8±7 years), 2. 3A/4A - 3A/3A (51.7±8 years),

3A/4A (50.9±8 years), 4A/4A (52.5±7 years), 3.T-1370G - TT (51±8 years), TG (50.8±8.6 years), GG (50±4.5 years). Comparing the ages of ESRD in patients with different 3A/4A and K198N haplotypes we found significantly lower age of ESRD (47.1 ±5.9 years) in the carriers of 4A allele in combination with 198N allele than in the carriers of 4A allele homozygous for K198 allele (52.9±9.4 years) (t-test, $p< 0.01$) and in the carriers of 198N allele homozygous for 3A allele (53±9.8 years) (t-test, $p< 0.05$).

We found deleterious effect of the combination of 4A allele and 198N allele of EDN1 gene was observed in ADPKD individuals.

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P1062. Decreased frequency of the TNF2 allele of TNF- α -308 promoter polymorphism is associated with lacunar infarction

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Background and Purpose: Enhanced release of proinflammatory cytokines may contribute to the pathogenesis of stroke. We examined whether G to A promoter polymorphism in the tumor necrosis factor- α gene at position -308 affects the stroke risk.

Methods- We genotyped 336 patients with ischemic stroke and 273 healthy controls for this polymorphism. We divided patients into different groups by both modified TOAST and Oxfordshire Community Stroke Project (OCSP) classification and determined the allele frequency in every group.

Results- Patients with ischemic stroke had a significantly ($p<0.001$) decreased (0.115) frequency of the -308 A (TNF2) allele compared to the healthy controls (0.196). When patients were classified according to a modified TOAST classification, reduction of the TNF2 allele was found to be restricted to the small vessel pathology, lacunar infarction group (allele frequency: 0.068, $p=0.003$ compared to the healthy controls). The result was confirmed by OCSP classification, where the TNF2 frequency in lacunar infarct (LACI) patients (0.065) was significantly ($p=0.002$) decreased.

Conclusions- Our results suggest TNF2 allele have a protective effect against the development of lacunar subtype of ischemic stroke.

P1063. No evidence of association of CXCL2 haplotypes with the development of sepsis

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Sepsis is the most common cause of acute lung injury (ALI). It has been shown that inflammatory and immune responses, cell proliferation, chemotaxis and blood coagulation are the five key biological processes involved in the development of sepsis and its complications. CXCL2 (MIP-2 alpha) has been recently catalogued as a candidate gene for ALI based on gene expression profiles derived from multiple animal models of lung stress. The encoded protein acts as a potent chemoattractant, and the abolishment of its effects leads to lung injury. In this study we have analysed the ~2.1 kb sequence of the CXCL2 gene in 66 septic patients and 364 unrelated population-based controls from the Canaries (Spain). We first measured linkage disequilibrium in 86 chromosomes using two previously described SNPs (rs3806792 and rs9131) and a insertion/deletion of [ATT] motif (rs5859414), and found no evidence of historical recombination in the region. We then used one of these SNPs (rs3806792) along with a newly described polymorphic short tandem repeat (D4S3454) to obtain known phased haplotypes and analysed their association with the disease. We tested the overall haplotype frequency distribution between cases and controls and found no statistically significant differences. These preliminary results suggest that CXCL2 genetic variants may not

contribute substantially to the development of sepsis.
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P1064. LBP genetic variants and susceptibility to sepsis

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Sepsis is the most common cause of acute lung injury, organ dysfunction and death in critically ill patients. Previous association analyses using candidate genes support that variants in the genes involved in inflammation play an important role in the development of sepsis. Prior studies used *LBP* (the protein recognises Gram-negative bacteria endotoxin) as a candidate gene. However, the positive results found for a variant in the earliest study have not been replicated. Our aim in this work is to study the association of *LBP* gene with sepsis using a "haplotype tagging" approach. On the basis of their gene distribution and frequency, 11 SNPs were selected and assembled into two SNaPshot multiplex reactions for the analysis. Four of them were tagging identifying more than 90% of European haplotypes. After haplotype reconstruction with PHASE 2.1 program, we assessed whether differences between cases and unrelated population-based controls from the Canaries (Spain) exist through an omnibus likelihood ratio test statistic for the overall haplotype frequency profile, with significance empirically determined via randomisation tests. Our results are suggestive of the existence of sepsis susceptibility alleles on one or some sets of chromosomes exhibiting the allelic patterns of haplotypes studied.

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P1065. Vitamin D receptor allele combinations and *FokI* polymorphism influence genetic susceptibility to type 1 diabetes in Dalmatian population

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INTRODUCTION: Vitamin D and its receptor gene (VDR) have immunomodulatory properties and influence insulin secretion. Recent data indicate VDR polymorphisms to be associated with type 1 diabetes mellitus (T1DM) and therefore we studied *FokI* polymorphism in exon 2.

MATERIALS AND METHODS: 134 individuals with T1DM and 232 control subjects from the Dalmatian population of South Croatia were examined. *FokI* genotyping was performed by PCR amplification followed by *FokI* endonuclease restriction. Data were analysed using the chi-square test. On the basis of our previous research, haplotype analysis of VDR polymorphisms *Bsml*, *Apal* and *TaqI* and their association with susceptibility to T1DM have been investigated. Estimates of haplotypes were made using the statistical program EH+.

RESULTS: *FokI* polymorphism showed unequal distribution ($p=0.0049$) between T1DM cases and controls indicating ff genotype to be associated with development of T1DM and Ff genotype to be a protective one. *Bsml*, *Apal* and *TaqI* polymorphisms were found to be in strong linkage disequilibrium (LD) ($p<0.0001$). Bf haplotype was observed 11 times in controls and not once in T1DM individuals ($p=0.0007$) and therefore revealed its possible protective role. No significant LD between *FokI* and any other polymorphism was detectable but, nevertheless, bf haplotype was significantly more frequent in T1DM patients ($p=0.0168$) while the aF haplotype was observed more times in controls ($p=0.0413$).

CONCLUSIONS: Our findings indicate that VDR *FokI* polymorphism and several VDR allele variants are associated with susceptibility to T1DM in the Dalmatian population.

P1066. Genetic variants in both cysteinyl leukotriene 1 and 2 receptors are independently associated with atopy and asthma in a Tristan da Cunha isolate.

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Asthma is a complex disorder with heterogeneous etiology, while atopy is a well-defined phenotype of immune hyperactivity that often underlies asthma. The genes encoding the cysteinyl leukotriene 1 (CysLT₁ at Xq13.2) and 2 (CysLT₂ at 13q14) receptors have been implicated in atopy, in part because they bind the cysteinyl leukotriene ligands that mediate atopic asthma, and they are targets for anti-leukotrienes used to treat asthma. We analyzed the association of single nucleotide polymorphisms (SNPs) of CysLT₁ (p.G300S) and CysLT₂ (p.M201V) with either asthma or atopy phenotype. We genotyped the Tristan da Cunha isolate with an excess prevalence of atopy (47% of 112 subjects), and smaller cohorts of asthmatics (n=25) and non-asthmatics (n=25) from the Boston area. The frequencies of the 300S and 201V variants were 15% (25/167 X chromosomes) and 13% (30/224 alleles) in the Tristan da Cunha sample, and 4% (3/70 X chromosomes) and 0% in the Boston sample, respectively. While the trend in the Boston sample did not reach significance, both CysLT₁ and CysLT₂ SNPs were highly correlated with atopy in the Tristan da Cunha sample ($r=.35$, $p<.001$ & $r=.33$, $p<0.001$, respectively). However, only the CysLT₂ variant was predictive of asthma ($r=.21$; $p=0.045$). All double heterozygotes were atopic, and further analysis of joint genotype is consistent with an additive model ($r=.45$; $p<.001$), whether corrected for age, gender and smoking, or not. This is the first description of association between CysLT_{1/2} genotypes and atopy/asthma phenotypes. Further testing in larger panmictic populations is warranted.

P1067. Association of CARD15 gene variants with pediatric inflammatory bowel diseases

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Three major polymorphisms of CARD15 gene (R702W, G908R, L1007fsinsC) have been associated with Crohn's disease (CD), but not with ulcerative colitis (UC), in different Caucasian adult populations. We analysed the three variants in 198 pediatric Italian patients affected by inflammatory bowel disease (IBD), including 112 with CD and 86 with UC, with an onset at ≤ 18 years. As a control group, 115 unaffected individuals were analysed. Comparison of allele frequencies between patients and controls demonstrated an independent association for all three variants with CD, but not with UC. After combining the three polymorphisms, 39.3% of CD patients carried at least one CARD15 variant compared to 5.2% of controls ($P<10^{-9}$; $OR=11.75$; 95% CI=4.75-29.06). Eleven CD children (10.1%), but only one UC and no control subjects carried two CARD15 variants (homozygotes or compound heterozygotes). The combined frequency of CARD15 variants was higher also in UC children compared to controls (14% vs 5.2%; $P=0.029$; $OR=2.95$, 95% CI=1.06-8.2). Analysis of genotype-phenotype correlation did not find any significant clinical difference between CARD15 positive and negative patients in both CD and UC groups. The present study shows that the major CARD15 variants are

strongly associated with pediatric onset CD in Italy. A possible role for these polymorphisms in pediatric UC is also supported; however, this association needs to be confirmed in larger cohorts. Present results do not predict a role for CARD15 genotypes in the clinical presentation of pediatric IBD patients.

P1068. The detection of genotyping errors and pseudo-SNPs via deviations from Hardy Weinberg equilibrium

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Genotype error can greatly reduce the power of a genetic study. For family data, genotype error can be assessed by examining marker data for non-Mendelian inconsistencies, closely linked markers for double recombination events and consistency of duplicate genotypes. For case-control data, duplicate samples are genotyped, and controls are tested for deviations from Hardy-Weinberg Equilibrium (HWE). Although genotyping errors can cause deviations from HWE, these deviations are usually small, and the power to detect them is low except for high rates of genotyping error and/or large sample sizes. An additional problem is that even when deviations from HWE are detected for marker loci, without additional experimentation it is not possible to unequivocally implicate genotyping error as the cause. The power and sample sizes necessary to detect deviations from HWE for SNP data was examined for a variety genotyping error and pseudo-SNP models. For the majority of the genotyping models examined, the power is poor to detect deviations from HWE. For example, for 1,000 controls, if an allele with frequency of 0.1 fails to amplify for 28% of the heterogeneous genotypes producing a sample error rate of 0.05, the power is 0.51 to detect a deviation from HWE at an alpha level of 0.05. On the other hand, the detection of deviations from HWE for pseudo-SNPs (paralogous and ectopic sequence variants) for the majority of models examined produced a power of >0.8 for sample sizes as small as 50 individuals.

P1069. SimPed: A simulation program to generate haplotype and genotype data for pedigree structures

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With the widespread availability of SNP genotype data, there is greater interest in analyzing pedigree haplotype data. For microsatellite markers due to their physical distance intermarker linkage disequilibrium is usually low; however, for dense maps of SNP markers, there can be strong linkage disequilibrium between marker loci. Linkage analysis (parametric and nonparametric) and family-based association studies are currently being carried out using dense maps of SNP marker loci. Monte Carlo methods are often used for both linkage and association studies; however, to date there are no programs available which generate haplotype and/or genotype data consisting of a large number of loci for pedigree structures. A program, SimPed has been developed to quickly generate haplotype and/or genotype data for pedigrees of virtually any size and complexity. Marker data either in linkage disequilibrium or equilibrium can be generated for > 10,000 diallelic or multiallelic marker loci. Haplotypes and/or genotypes are generated for pedigree structures using specified genetic map distances and haplotype and/or allele frequencies. The data generated by the SimPed program is useful for a variety of analysis purposes, including evaluating methods that estimate haplotype frequencies for pedigree data and estimating empirical p-values for linkage and family-based association studies.

P1070. The influence of interleukin-10 (IL-10) promoter polymorphism on the occurrence of non Hodgkin Lymphoma (NHL) in subjects infected with Hepatitis C Virus (HCV).

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HCV along with chronic liver disease is also considered a causative agent of other clinical pathological conditions which testify the possible direct pathogenic role of the virus in several different cell types including hepatocytes and leukocytes. Prevalence of HCV is significantly higher

also in patients suffering with NHL and, all around the world, it was confirmed except for patients studied in North Europe and some areas of North America. In Italy, different groups showed prevalence ranging from 15 to 30%. The goal of this paper is to establish if a polymorphic gene encoding for cytokine could be a predisposing factor for this condition. To do this, we analyzed the distribution of the polymorphism of IL-10 -1082 G/A in 63 patients, not infected with HCV, with NHL (NHL/HCV-) and in 50 patients, infected with HCV, with chronic active hepatitis, with NHL, (NHL/HCV+). In this study, for the first time we show that regardless of age, sex, virus genotype and/or severity of chronic liver disease a significant prevalence of IL-10_{-1082 GG} genotype seems to influence the occurrence of NHL in HCV infected patients. In fact the distribution of the IL-10_{-1082 G/A} polymorphism was different between NHL/HCV+ and NHL/HCV- patients ($P=0.028$). The frequency of the IL-10_{-1082 G} allele ($P=0.019$) and the frequency of the IL-10_{-1082 GG} genotype against overall genotypes (IL-10_{-1082 GA/AA}) were significantly higher in NHL/HCV+ patients as compared with NHL/HCV- patients ($P=0.014$).

P1071. N-methyl-D-aspartate receptor subunit NR1 gene (GRIN1) and temporal lobe epilepsy: no evidence of association analysis for genetic variant in promoter region of the gene

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Glutamate is the principal excitatory neurotransmitter in the brain. Excitatory amino acids are very important in the synaptic transmission of the brain, and for this reason does not surprise that such excitatory pathways is involved in the onset and propagation of epileptic seizures. Recently, in epileptic animal models has been shown that increased excitability might be due to an abnormal glutamatergic transmission involving altered properties of ionotropic N-methyl-D-aspartate (NMDA) receptors.

Here, we aim to assess if the human non lesional temporal lobe epilepsy (TLE) is associated to changes in the subunit NR1 of the GRIN1 receptor which is the common subunit of the NMDA receptor. We screened single nucleotide polymorphisms (G1001C) of the upstream region using a case-control study. The alleles distribution of the G1001C polymorphism were analyzed in 266 Italian patients with non-lesional TLE (146 women and 120 men; mean age 47.3±17.8) and in 374 healthy controls (182 women and 192 men; mean age 49.2±21.9) matched for age, sex and ethnicity. The DNA study was performed using a PCR amplification followed by digestion with a restriction endonuclease.

No statistical significant differences in the 1001G/C genotype and allele frequencies between patients with temporal lobe epilepsy and controls ($p=0.053$ and $p=0.032$, respectively) were found. Neither association was found in sex, age at onset and duration of disease after dichotomization of the data in terms of carrier or non-carrier for C allele.

In conclusion, our results do not suggest any association between the GRIN1 polymorphism (G1001C) and non lesional temporal lobe epilepsy.

P1072. Association of paraoxonase 1 gene polymorphisms with Type 2 diabetes mellitus

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Background: Low antioxidant activity of paraoxonase (PON1) in patients with Type 2 diabetes mellitus (DM), where an increase in oxidative stress was detected, has been traced, in part, to polymorphisms within promoter and coding regions.

Aim: To investigate the association of the four single-nucleotide polymorphisms (SNPs) of PON1, Met-Leu 55 (M55L), Gln-Arg 192 (Q192R) in coding and in promotor region (-107C/T, -907G/C) with DM.

Design and methods: Case-control association study. Distribution of the SNPs was determined by PCR-RFLP. The allele and genotype frequencies were calculated in DM (n=109) diagnosed by the WHO

criteria and in healthy controls (n=70). There were no differences between these groups in gender and age distribution. Following biochemical parameters were measured. Fasting plasma glucose (FPG), concentration of triglycerids (TG), LDL - cholesterol (LDL-C), glycated hemoglobin (FPG 9,29±3,09 mmol/l, TG 1,91±0,32 mmol/l, LDL-C 3,22±0,78 mmol/l, glycated hemoglobin 6,65±1,76 in DM vs. FPG 4,95±0,49 mmol/l, TG 1,43±0,63 mmol/l, LDL-C 3,00±0,66 mmol/l in controls). Normal urinary excretion of albumin was found in all patients.

Results: The allele frequencies of L55M were L= 0,583, M= 0,417 in DM vs. L= 0,850, M= 0,150 in controls, in Q192R were Q= 0,849, R= 0,151 in DM vs. Q= 0,543, R= 0,457 in controls. There were detected statistical significant differences in allele and genotype frequencies between studied groups in L55M (P=0,01) and Q192R (P= 0,01), whereas polymorphisms in promotor region differed statistically nonsignificant.

Conclusion: These data indicate that PON1 SNPs in coding area could be regarded as genetic factors associated with DM.

P1073. Performance evaluation of the SNPlex Genotyping System using population validated SNPs

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In addition to producing highly accurate and reliable data, a high-throughput SNP genotyping system must offer speed and flexibility, paired with low cost. We have recently introduced the SNPlex™ Genotyping System that utilizes multiplexed OLA/PCR and capillary electrophoresis to genotype SNP information encoded in genomic DNA. In each multiplex reaction, up to 48 SNPs are interrogated in parallel, using a set of SNP-specific ligation probe pools. After purification, all specific ligation products are amplified simultaneously with one pair of universal PCR primers. Biotinylated amplicons are captured in streptavidin coated microtiter plate wells. A set of universal fluorescently labeled probes, called ZipChute™ reagent, is hybridized to single-stranded amplicons. After selective hybridization, the ZipChute probes are eluted for subsequent detection on Applied Biosystems 3730/xl and 3130xl Analyzers. The GeneMapper® Software determines the SNP genotypes, which are displayed in cluster plots with associated genotype data. We will present data generated by utilizing a simplified SNPlex System assay workflow that validate the SNPlex System for high throughput SNP genotyping. Accuracy and precision data generated by different sites, obtained both with 3730/xl and 3130xl Analyzer platforms, will be shown for a 48-plex SNP set, the SNPlex System Control Pool. This set consists of an optimized ligation probe set that addresses 48 population validated SNPs. This control pool together with a genomic DNA panel is designed for use in quality control, trouble shooting, and training purposes.

P1073. The role of vascular and hormonal gene variants in migraine susceptibility

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Migraine is a common and debilitating neurological disorder characterised by headpain, nausea, vomiting, photophobia and often severe, neurological disturbances. Although calcium channel and ATPase gene variants have been implicated in the rare familial hemiplegic migraine sub-type, the exact number and identity of genes involved in the more common types of migraine have yet to be defined. Genetic linkage studies in our laboratory have so far revealed migraine susceptibility regions on chromosomes 19, X and 1, emphasising the heterogeneity of the disorder. In order to identify the molecular basis of migraine, neurotransmitter-related pathways have been the main focus of studies. However vascular and hormonal disturbances also occur in migraineurs, as highlighted by alterations in cerebral blood flow and hormonal triggers of migraine. We have recently investigated a number of genetic factors involved in these functions. These studies have provided evidence implicating variants in the methylenetetrahydrofolate reductase (MTHFR) and angiotensin I-converting enzyme (ACE) genes, as interacting determinants of migraine, that increase total migraine risk by ~2-fold and migraine with aura risk

by ~3 fold. We have also investigated a number of hormonal gene variants. These studies have implicated variants in both the estrogen receptor and progesterone receptor genes. Furthermore, interaction analysis revealed that individuals who carried at least one copy of both hormone receptor susceptibility genotypes were even more likely to suffer from migraine, indicating that these genes also appear to act synergistically. These overall results indicate that migraine is a complex polygenic disorder, involving multiple and possibly interacting gene components.

P1074. Hypertension with and without metabolic syndrome presents as distinct clinical and genetic entities in French-Canadians

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Hypertension is generally perceived as a constitutive attribute of the metabolic syndrome, though not a requisite one in either of the definitions by the World Health Organization or the National Cholesterol Education Program's Adult Treatment Panel III. While hypertension without metabolic syndrome has received little attention recently, its genetic determinants and cardiovascular outcomes may be distinct from hypertension within the context of metabolic syndrome.

In 120 hypertensive French-Canadian families from the Saguenay-Lac-St-Jean region of Quebec, we identified and compared those with a high density (>50%) of metabolic syndrome (HDMS, n=25) vs. families with no metabolic syndrome in propositus sibships (LDMS, n=23). Using a method of layered founders, we assessed the separability of hypertensive HDMS and LDMS families in terms of specific ancestry. We compared > 40 hemodynamic, metabolic and anthropometric traits and their heritability. The LDMS hypertensives showed distinct features in comparison to hypertensives in HDMS families, e.g. significantly lower volume of extracellular water (19.0±0.5 vs. 16.2±0.5 L, p=0.0001) and higher Na⁺/K⁺ cotransport in erythrocytes. Heritability estimates suggested higher heritability of blood pressure, extracellular water and several metabolic traits in LDMS families. At the depth of 14 layers (generations), the ancestors of LDMS families showed a higher specificity of genetic contribution to the recent generation in contrast with HDMS families (75% vs. 55%). In summary, hypertension with and without features of metabolic syndrome constitutes distinct entities in terms of genetic and clinical characteristics, possibly with specific cardiovascular outcomes.

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P1075. The effects of complex unaccounted pedigree structure on homozygosity mapping

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Genetically isolated populations have proven to be a useful tool in mapping genes underlying complex human disease. One of the most powerful tools, which could be applied for gene identification in such populations, is homozygosity mapping. When performing homozygosity mapping, one may typically account only for a few of the shortest loops, in part because more extended genealogic information may be missing, or due to computational complexities arising in analysis of larger pedigrees. Ignoring part of pedigree information might lead to false positive findings. We evaluated the effect of underestimation of the inbreeding on type I error of homozygosity mapping using heterogeneity LOD scores. In general, the inflation of type I errors is sensitive to the extent to which true inbreeding is underestimated and to marker allele frequencies. We investigate type I errors using simulated data and real data on Alzheimer disease patients identified in a recent genetically isolated population. Using this data, we show which scenario may lead to a real hazard in gene-identification. We

work out recommendation on how to avoid inflation of type I error and over-estimation of obtained LOD scores.

P1077. The E-selectin S128R polymorphism in patients with ankylosing spondylitis from Romanian population

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As an active participant in inflammatory processes, the vascular endothelium produces E-selectin in response to proinflammatory cytokines. The E-selectin gene S128R (serine to arginine) polymorphism has not been investigated yet in relation with ankylosing spondylitis (AS).

We have tested a limited number of AS patients (N=35) and an ethnically matched control group (N=40) from Romanian population for S128R polymorphism in E-selectin gene. In the case group, 24 (68.6%) individuals exhibited wild type E-selectin 128S genotype, 7 (20%) were heterozygous S128R and 4 (11.2%) were homozygous for the 128R allele. In the control group, the distribution was 26 (65%) homozygous 128S, 13 (32.5%) heterozygous S128R and 1 (2.5%) homozygous 128R.

The distribution of the E-selectin genotype in our control group showed Hardy-Weinberg equilibrium, which verified that the control group was statistically appropriate, and that the genotyping protocol was error-free.

The genotype distribution in the AS group was statistically significant different ($\chi^2=5.74$) from the Hardy-Weinberg equilibrium.

Disease association with S128R was assessed by single-locus and single-locus allele based χ^2 tests for a 3x2, respectively 2x2 contingency table, to compare each genotype/allele frequency in patients with AS versus control individuals. No significant association was found in either situation.

The Hardy-Weinberg disequilibrium observed in AS group could be produced by random sampling errors due to small population size (estimated frequency of AS in general Caucasian population is 0.1-0.2%).

P1078. Contribution of polymorphisms Ile50Val and A1188C in *IL4RA* and *IL12B* genes into predisposition to hepatofibrosis development in patients with viral hepatitis

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High prevalence of viral hepatitis and severe complications of the disease such as cirrhosis and hepatocellular carcinoma, cause the interest to study genetic basis of susceptibility to viral hepatitis. Genetic status of the host organism is one of the main factors which determine individual reaction to the viral infection and particularities of disease course. We studied the association of polymorphisms Ile50Val in *IL4RA* gene and A1188C in 3'-UTR region of *IL12B* gene with clinical features of chronic viral hepatitis and extent of hepatofibrosis. The case group consisted of 61 patient with chronic hepatitis. A sample of Tomsk city inhabitants was used as a control group (N=128). The genotyping was done by PCR-restriction analysis. There were no significant differences in genotypes and allele frequencies between cases and controls. Also, no association of A1188C polymorphism in *IL12B* with disease course was identified. However, there was difference in genotype distribution for *IL4RA* polymorphism in subgroups of patients divided by different extent of fibrosis. From one side, there was increasing of the frequency of homozygotes Ile/Ile and Val/Val in the group without fibrosis, and from other side, there was accumulation of Ile/Val heterozygotes along with fibrosis progression, from 7.1% in the group without fibrosis up to 47.6% in initial fibrosis ($p=0.035$) and 57.7% in moderate and profound fibrosis ($p=0.004$). This finding suggests that the carriers of Ile/Val genotype for *IL4RA* have increased risk for hepatofibrosis development while being affected by viral hepatitis.

P1079. PED 5 pedigree drawing software

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PED is a fast interactive pedigree drawing software for Windows 98 (R) and above. PED was introduced at the ESHG Conference in 1997 and now has a continuously growing user community with researchers and clinicians in over 30 countries worldwide. PED complies with the "Recommendations for standardized human pedigree nomenclature" proposed by the PSTF (Bennet RL et al, Am J Hum Genet 56:745-752, 1995). Apart from fully sizable printed output, pedigrees can be exported as Windows metafiles (WMF) to virtually any Windows Office or drawing program.

Among other enhancements, PED 5 now can fetch family information from a data file with LINKAGE or CSV (comma separated values) format, where each line describes an individual by the pedigree ID, the individual's ID, the IDs of his/her father and mother, the gender, the phenotype or affection status, and any other data related to the individual (like haplotypes, or clinical characteristics). Imported pedigrees can virtually be of any size; also descendants with a second, third, or even tenth partner or any descendants of the probands' ancestors will be correctly displayed. Currently, marriage loops are not handled; they must be edited in the edit window. Pedigree charts can also be exported to these standard file formats. With each open/edit/save cycle, all IDs will be preserved. So pedigree data can easily be imported into a spreadsheet, a database, or further be used in linkage calculations or risk assessment. A demonstration program is available at www.medgen.de/ped.

P1080. The *MTHFR* C677T variant is associated with an increased risk of migraine with aura in Canarian patients.

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Migraine is a complex neurovascular disorder that affects approximately 12% of the Caucasian population. Although the pathophysiological events that underline migraine are not fully understood, migraine neurobiology may be explained as the result of disturbances in neurological pathways and cerebral circulation. High serum homocysteine levels have been shown to produce endothelial injury, altering the cerebrocirculatory system. The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) enzyme is involved in homocysteine metabolism. The *MTHFR* C677T genetic variant has been correlated with a reduced enzyme activity and hyperhomocysteinemia.

In this study we determined the prevalence of the *MTHFR* C667T polymorphism in migraineurs and test its association with migraine susceptibility using an unrelated case-control design. Seventy four patients with migraine headache (50% with aura, MA, and 50% without aura, MOA) and 118 non-affected controls were recruited from the same geographical area (Canary Islands, Spain) and genotypes for the *MTHFR* C677T were determined by PCR-RFLP.

Our results show that the *MTHFR* 667T allele was over-represented in the migraine group compared to the control group, principally among the MA subtype (43.2% vs 29.7%) ($\chi^2=3.98$, $p=0.04$). A comparison of the genotype distribution showed a higher incidence of the homozygous transition (T/T) in the migraine group and specifically in the MA group. The T/T genotype of the *MTHFR* gene in the Canarian patients studied was associated to an increase risk of suffering migraine (OR= 1.6 95% CI 1.05-2.49, $p=0.02$), particularly MA (OR= 1.8 95% CI 1.05-3.09, $p=0.03$).

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P1081. Polymorphisms of mitochondrial nuclear-encoded complex II and Citrate Synthase genes in idiopathic male infertility

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Previous studies have shown a correlation between sperm cell concentration in the ejaculate and nuclear-encoded [Succinate dehydrogenase (SDH) complex or complex II and citrate synthase (CS)] mitochondrial enzyme activities [Ruiz-Pesini et al., (2000) Clinica Chimica Acta 300, 97-105]. From these data, we postulate

that enzymatic expression should be decreased in severe sperm impairment and investigate if this decrease could be a consequence of the presence of sequence variants on these genes.

An exhaustive mutational study of the five genes was performed using the SSCP/HD technique followed by sequencing of abnormal migrating patterns in 44 infertile men with a phenotype of non-obstructive azoospermia or severe oligozoospermia (<5 million sperm per mL) and 46 fertile men as the control group. Several sequence variations in these genes were identified: 21 in SDHA (13 in coding regions), 4 in SDHB (two of them in exonic sequences), 4 in SDHC (one in a coding region), 5 in SDHD (three of them in coding regions), and 5 in CS (one in an exon). Five of these nucleotide changes produced amino acid substitutions. The frequency of nucleotide variations showed no statistically significant differences between infertile and fertile groups. These preliminary results suggest that no particular allele or haplotype in these genes appears to be associated to male infertility and that the activity of mitochondrial enzymes may be transcriptionally and/or translationally regulated. New SNPs identified should be useful in segregation and linkage disequilibrium analyses in families affected by other disorders caused by these nuclear encoded mitochondrial genes.

P1082. Homozygosity mapping of an Usher-like syndrome to chromosome 15q21.3-q23

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Mutations in 7 different genes have so far been identified in Usher syndrome and 4 loci have been mapped. Three clinical types are distinguished.

In 1996, an autosomal recessive Usher-like syndrome was reported in two branches of a large, consanguineous family from Denmark. A common ancestor born in 1702 was identified.

The syndrome is characterised by congenital cataract, which distinguishes it from other Usher types, severe and progressive hearing impairment with onset from 8 to 20 years, adult-onset retinitis pigmentosa and vestibular dysfunction. This is the only reported family with the syndrome.

In order to try to identify the causative gene, we performed homozygosity mapping in five affected members of the family with 400 polymorphic microsatellite markers with an average spacing of 9.2 cM. The disease was mapped to an 11.8 Mb interval on chromosome 15q21.3-q23, between markers D15S648 and D15S1025. The region contains around 100 known genes. By cDNA sequencing, four candidate genes in the region were excluded as responsible for the disease.

P1083. Apolipoprotein AV gene SNP determination in patients with high triglyceride levels

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Objective: A new gene designated apolipoprotein A-V (APOAV) has been identified in the APOAI/APOCIII/APOAV gene cluster. The aim of our study was to examine the SNPs frequencies in a group of patients with extreme triglyceride levels compared to controls with normal lipid ones. **Methods:** The patient group consisted of 132 unrelated individuals, in the control group 59 people were screened. Five SNPs were tested (SNP3-rs662799, rs651821-Kozak, rs1729410, rs4938312, rs648450) and variability of the exon 1 and a putative TATA box together with the DR1 and IR8 elements in the promoter ApoAV region was screened. **Results:** The frequency of carriers of SNP3 minor allele (7 homozygotes and 41 heterozygotes) were much higher in patients group compared to the population sample. On the other hand no homozygotes and 3 heterozygotes only were determined in the control group. The same allele distribution was observed for the SNP rs651821-Kozak. SNP rs1729410 did not show any conclusive linkage association evidence. We did not find any polymorphism in the case of rs4938312 and rs648450. Neither mutation nor polymorphism were determined in the exon1 or the promoter region. **Conclusions:** Our study verified the association between the SNP3 and SNP-Kozak minor allele in the APOAV gene and extreme triglyceride levels.

Presented results support the assumption, that ApoA5 belongs to the most important genetic determinants of plasma triglycerides detected so far.

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P1084. The interaction between Metabolic Syndrome and PON1 polymorphisms increases the risk of coronary artery disease

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Background. Serum paroxonases play an important role in anti-oxidant defences and prevention of atherosclerosis. Metabolic syndrome is a clinical condition associated with increased oxidant stress and cardiovascular mortality. Two common polymorphisms of PON1, Leu55Met and Gln192Arg, have been suggested to modulate cardiovascular risk.

Methods. 915 subjects were subjected to angiographic documentation of coronary artery vessels: 642 were classified as atherosclerotic (CAD) and 273 as non atherosclerotic (CAD-free). 224 subjects met the diagnostic criteria of metabolic syndrome. All subjects were genotyped for the two PON1 polymorphisms.

Results. A significant interaction between metabolic syndrome and both PON1 polymorphisms in determining CAD risk ($p<0.05$) was observed. The 55Leu and the 192Arg alleles, associated with reduced protection against lipid peroxidation, were associated with CAD only in the metabolic syndrome subgroup. Subject with metabolic syndrome and both 55Leu and 192Arg alleles had significantly increased risk (OR 9.38, 95%CI 3.02-29.13 after adjustment by multiple logistic regression) relative to subjects with 55Met/Met-192Gln/Gln genotype. **Conclusion.** PON1 polymorphisms modulate coronary artery disease in metabolic syndrome patients.

P1085. Further evidence for a genetic heterogeneity of Mal de Meleda

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Mal de Meleda (MDM) (MIM 248300) is a rare inherited skin disorder which belongs to a clinically and genetically heterogeneous group of palmoplantar keratoderma (PPK). The disease has a world-wide distribution mainly in the Mediterranean population. Clinically, MDM is characterized by erythema and hyperkeratosis extend to the dorsal face of hands and feet. The gene responsible for MDM, *ARS* (component B)-81/s, has been mapped on chromosome 8qter using homozygosity mapping. The *ARS* gene encodes for the SLURP-1 protein (Ly-6/uPAR related protein-1). Various mutations within the *ARS* gene have been identified to underline MDM disease in different populations. We have recently demonstrated that three different homozygous mutations (82delT, C77A, C99Y) are responsible for MDM disease in 17 patients belonging to 8 unrelated consanguineous families from Northern Tunisia. We report here, one Tunisian family with three siblings presenting with classical clinical features of MDM. No mutation was detected by direct sequencing of all three exons and flanking intronic region of the *ARS* gene for the three patients. Furthermore, linkage analysis, by genotyping all family members, using microsatellite markers, excluded the *ARS* gene region. Genetic heterogeneity is a likely explanation for our findings, as this has been already hypothesized by other authors. A genome-wide search for the locus of the present phenotype is in progress.

P1086. Analysis of eNOS, ACE and MTHFR genes polymorphisms in placental insufficiency.

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Recently, significant associations of endothelial nitric oxide synthase (*eNOS*), angiotensin converting enzyme (*ACE*) and methylenetetrahydrofolate reductase (*MTHFR*) genes polymorphism with thrombophilia at pregnant, recurrent spontaneous miscarriages and preeclampsia in women of different populations were reported (Tempfer et al, 2001; Buchholz et al, 2003; Vefring et al, 2004; Lin et al, 2005). These loci are located on different chromosomes and encode products involved into various metabolic pathways leading to obstetrics pathology. Therefore, we studied the *eNOS* VNTR polymorphism in 4 introne, *ACE* I/D polymorphism and *MTHFR* C677T polymorphism in women with to placental insufficiency (PI) from Bashkortostan (Russia). 78 women with PI induced gestational hypertension and 130 normotensive women with normal pregnancy were genotyped using PCR technique and subsequent enzyme digestion. We did not find significant association of the *eNOS*, *ACE* and *MTHFR* polymorphisms with PI, but there were differences in *ACE* genotype frequencies between women with gestational hypertension and normotensive women ($\chi^2 = 3.80$; $p = 0.049$).

Further studies are needed to confirm the *eNOS*, *ACE* and *MTHFR* polymorphisms with PI.

P1087. Association between GEFS+3 locus and GEFS+ syndrome in two affected Tunisian families

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Febrile seizure (FS) occur between 3 months and 6 years of age, and do not show evidence of defined cause. In FS, only five loci have been reported but no responsible gene has been identified so far. FS and epilepsy have been recently described in a syndrome called generalized epilepsy with febrile seizure plus (GEFS+). GEFS+ syndrome is an heterogeneous autosomal dominant disorder characterized by FS which persist after 6 years of age and/or by afebrile seizures. Four responsible genes of GEFS+ have been identified: *SCN1B*, *SCN1A*, *SCN2A* and *GABRG2* localized on chromosomes 19q13.1, 2q24 and 5q31.1-q33.1 respectively.

The aim of this report is to search for a linkage and/or association between the known FS and GEFS+ loci and GEFS+ syndrome in affected Tunisians families.

A total of 14 patients with GEFS+ and 55 controls belonging to two Tunisian families affected with GEFS+ syndrome were studied. Genetic analysis of genomic DNA has been performed using microsatellite markers spanning the FS and GEFS+ loci. Statistical analysis has been realized by intrafamilial association test (FBAT) and by parametric linkage (LOD scores) and non-parametric linkage (NPL) tests. In addition, a transmission disequilibrium test (TDT) has been performed using the computer program GENHUNTER v 2.1.

The result of FBAT test revealed an association between GEFS+3 locus and GEFS+ syndrome in these two Tunisian families with a very significant *P* value. This association was also confirmed by results of TDT test.

P1088. Clinical Report of a Belgian family with Adams-Oliver syndrome and molecular analysis of 5 candidate genes.

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Adams-Oliver syndrome (AOS) is a rare congenital disorder defined by the combined presence of both limb truncation and scalp defects, frequently associated with underlying defects in the parietal bones. We present a Belgian AOS family with several affected individuals over 4 generations. Clinical symptoms included large areas of alopecia on the vertex of the skull and serious limb reduction defects with large phenotypic variation between the affected family members.

Although AOS is clearly genetic and mostly inherited as an autosomal dominant trait, no disease causing gene has been identified so far. We therefore selected a number of candidate genes for AOS based on the phenotype of knockout mice, and/or clinical overlap with other human diseases. Using sequencing and linkage analysis in 10 AOS families, the *MSX1*, *CART1*, *P63* (*P73L*), *RUNX2* and *HOXD13* genes

were analyzed. We could not detect any disease causing mutations, but further investigation is ongoing to identify the genetic cause of AOS in these families.

P1089. A second locus for autosomal recessive nonsyndromic optic atrophy

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Nonsyndromic optic atrophy, when familial, is usually transmitted in an autosomal dominant pattern. Recessive optic atrophy is rare and is usually associated with multisystemic disease involving the central nervous system and other organs. Recently, a locus for isolated autosomal recessive optic atrophy (ROA1) has been mapped to chromosome 8q21-q22. We investigated a novel consanguineous family of Moroccan origin with nine children. Three of them were affected with isolated optic atrophy. They presented with moderate to severe loss of visual acuity, temporal optic disc pallor and color vision deficits. Two sibs reported subnormal vision since infancy. The third was initially suspected of Leber optic atrophy because of a sudden loss of visual acuity at age 16 years. No mutation was detected in mitochondrial DNA. Interestingly he was treated for subacute axonodemyelinating polyneuropathy at age 33. We performed a genome-wide search for homozygosity in affected siblings using an 11K SNP microarray chip (Affymetrix). A 10 cM homozygous region was found on chromosome 11. Our data show genetic heterogeneity within autosomal recessive optic atrophies, and suggest a causal link with polyneuropathy in one of our patients.

P1090. Alcohol dehydrogenase genes and susceptibility to Fetal Alcohol Syndrome in South African Coloured populations

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Mental retardation is a prominent feature of fetal alcohol syndrome (FAS) and is attributable to the teratogenic effects of *in utero* alcohol exposure in individuals with susceptible genetic variants. Possible candidate genes involved with the aetiology of FAS include alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH).

A case-control genetic association study was done to examine the roles of ADH1B, ADH1C and ADH4 in two Coloured populations from the Northern and Western Cape with a high prevalence of FAS. Single nucleotide polymorphisms associated with the genes were typed using PCR-based methods in the FAS children and controls.

Previous studies in the Western Cape suggested that the ADH1B*2 allele

confers protection against the development of FAS ($p=0.0036$), however this was not significant in the Northern Cape ($p=0.088$). Further preliminary studies on ADH1B, ADH1C and ADH4 have revealed significant associations where each of the alleles mentioned below appears to decrease the risk of developing FAS. Allele ADH1B*3 is significantly associated in the Northern Cape only ($p=0.043$); ADH1C*2 allele in both populations ($p=0.024$); and the 'T' allele of the rs1126670 polymorphic locus in ADH4 in the Western Cape only ($p=0.008$). These results show that the ADH genes do play a role in susceptibility to FAS in South African Coloured populations.

P1091. Homozygosity for a frequent and weakly penetrant predisposing allele at the RET locus in sporadic Hirschsprung disease

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Hirschsprung disease (HSCR), the most common malformation of the hindgut (1/5,000 live birth), is a neural crest derived malformation

characterised by the absence of enteric ganglia of variable length of the intestine. The genetics of HSCR is complex. Genetics and functional evidences support that the RET proto-oncogene is the major disease-causing locus. However, a mutation within the RET gene coding sequence could be detected in only 40% and 10-20 % of familial and sporadic cases respectively, despite families co-segregation with markers at the RET gene locus. These data suggesting a frequent hypomorphic allele(s) at the RET locus, led us and others to perform linkage disequilibrium mapping, using single nucleotide polymorphisms (SNPs) scattered along the RET genomic domain. We identified a predisposing haplotype located in the 5' region of the RET gene. In order to refine the mapping of predisposing allele(s) and to characterise its genetic behaviour, we used transmission disequilibrium test across the RET gene, in a series of HSCR cases divided according the presence/absence of a RET gene mutation, and the familiality (sporadic or multiplex). We observed highly significant over-transmission of a predisposing SNPs haplotype extending over 23kb from the promoter region to exon 2. Over-transmission was not significant when considering cases with RET gene mutation. Conversely, the majority of sporadic HSCR cases with no RET gene mutation showed homozygosity for the predisposing haplotype suggesting its major involvement and its dosage-sensitive effect on the RET signalling pathway in the commonest form of HSCR.

P1092. Linking *TGFA* gene to Cleft Lip with or without Cleft Palate

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The transforming growth factor alpha gene (*TGFA*, 2p13) has emerged as a promising candidate gene for orofacial clefting based on data from expression and allelic association studies. Assessment of different *TGFA* gene markers in a number of separate studies has resulted in association with CL/P phenotype as well as in negative results. Thus the question of if and how allelic variants of *TGFA* gene (eg. *TaqI* polymorphism) could contribute to the risk of clefting in children is still open.

In the present study biallelic *TaqI* marker (A1 allele is reference and A2 - variant, i.e. deletion of 4 bp in intron V) was analyzed in the sample of 51 unrelated case-parent triads, where the case was a child with an isolated non-syndromic cleft lip with or without cleft palate (CL/P).

The inheritance of *TaqI* marker alleles of the *TGFA* locus was studied by using PCR genotyping of the triads. The standard transmission/disequilibrium test (TDT) as described by Spielman et al. (1993) was used for statistic analysis. The paternity was confirmed in all families before TDT analysis. We found only 22 informative case-parent triads (i.e. heterozygous parent) out of 51 initially genotyped ones. We evaluated the presence of association between *TaqI* marker alleles in the *TGFA* gene and the risk of isolated CL/P. Our data provide no evidence of *TGFA* genotype in the *TaqI* site and susceptibility to cleft lip and/or palate within case-parent triads and there is no significant linkage disequilibrium with the A2 allele in *TaqI* site and CL/P phenotype.

P1093. Polymorphism in the non-coding region of human Mitochondrial DNA in Iranian diabetes type II patients

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The sequence in the first hypervariable segment (HVS-I) of the control region has been used as a source of evolutionary information in most phylogenetic analyses of mtDNA. Population genetic inference would benefit from a better understanding of the variation in the mtDNA coding region, but, thus far, complete mtDNA sequences have been rare. We determined the nucleotide sequence in the non-coding region of mtDNA from Iranian patients sequencing by direct sequencing of the D loop.

The D-loop region is a hot spot for mtDNA alterations and it contains two hypervariable regions (HVS-I and HVS-II). In order to identify polymorphic sites, and also to find out any possible variations in D-loop of diabetes typell patients, the complete non-coding region of mitochondrial DNA from 7 Azari type2 diabetic patients and 23 normal

controls from the same origin were sequenced. Alignment was made with the Cambridge Reference Sequence (CRS) and any differences recorded as single base substitution (SBS) numerical changes in C-tract (PCT), insertions and deletions .PCT changes were present in 52% compared to 71% diabetes patients. We found that polymorphism C150T was exist in 42.8% diabetes patients compared to normal cases (28.6%). Also variation in T16189C was found in 14.2% diabetes patients compared to our normal related population (28.6%). mtDNA mutations within the D-loop control region are a frequent event in Diabetes typell and may be an indicator of mtDNA instability.

P1094. Mutation analysis in Duchenne/Becker muscular dystrophy patients: identification of 11 novel mutations

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In approximately one third of Duchenne/Becker muscular dystrophy (DMD/BMD) patients the disease is caused by point mutations in the dystrophin gene on Xp21. Using SSCP-HD analysis followed by direct sequencing of the variants we have performed mutation analysis in a series of DMD/BMD patients after negative deletion screening. In addition to a series of known mutations, 11 novel mutations were identified: 3 nonsense mutations (Q393X, W1029X, W2479X), 1 missense mutation (S868G), 2 small deletions (4350delA, 5124_5127delGAAA), 1 duplication (5020_5021dupTT) as well as 4 splice-site mutations (357+1G>C, 1993-3C>G, 5922+5G>A, 7309+4G>A). In general nonsense mutations and other changes leading to a frameshift result in a truncated, non-functional dystrophin protein, causing the severe DMD form of the disease. Inframe and missense mutations, leaving the dystrophin partially functional, mostly lead to the milder BMD. The newly detected nonsense mutations as well as the deletions and the duplication confirmed this observation, while the missense mutation S868G was associated with a DMD phenotype. Predicting the effect of splice-site mutations is more difficult. The patients with 357+1G>C and 1993-3C>G suffered from DMD, while for those with 5922+5G>A and 7309+4 G>A the diagnosis was BMD. The mutations segregated with the disease and were not present in any unaffected male. Their pathogenic nature was inferred from these family data, as well as from the predictions by a splice-site finder. In order confirm pathogenicity of these mutations, RNA analysis will be performed.

P1095. Campora: an isolated population database for the study of cardiovascular traits

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The population of the small village of Campora in the Cilento area of South Italy was chosen as model of isolated founder population for the identification of allelic variants responsible of complex traits by means of genomic regions identical by descent (IBD) search. We have demonstrated that the population of this village derives from about fifty founders that contributed to the repopulation of the village after the plague of the 17th century. We attempted the description of the phenotype of each individual in the population by instrumental measurements of several parameters with particular attention to the cardiovascular system. We have assembled a database containing a 10,737-individual pedigree, spanning over 17 generations, and connecting all the 1400 living Campora's inhabitants with data derived from: the genome screening with a 1094- microsatellite map (average marker distance: 3.4 cM), blood tests, personal and familial past diseases, past and present drug therapies, diet, smoking habit and alcohol use, cardiovascular instrumental check up (Ultrasonography and ECG) to measure left ventricular interior dimension and mass, intima-media thickness of carotids, measurement of left ventricle volume changes, blood pressure and anthropological measurements. We will present our data on methodology of analysis and preliminary data of the search of loci responsible for cardiovascular disease

P1096. MDR1 C3435T Polymorphism and susceptibility to inflammatory bowel disease: lack of association in an Italian population.

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The C3435T multidrug resistance 1 (*MDR1*) gene polymorphism and homozygous TT genotype has been recently found to be overrepresented in ulcerative colitis (UC). However, further studies reported no evidence of this association with UC or Crohn's disease (CD). Aim of this study was to evaluate the frequency of C3435T *MDR1* polymorphism in UC and CD patients. 150 UC and 200 CD patients, and 300 healthy subjects were consecutively enrolled. All study participants were unrelated Caucasians of Italian origin. Genomic DNA was extracted from peripheral blood, genotyping was performed by PCR and *Sau3AI* restriction analysis. The results were confirmed by sequencing a limited number of samples using an automated DNA sequencer. Differences between cases and controls were compared by using a 2x2 contingency and χ^2 -test statistics. Odds ratios (OR) with 95% confidence intervals (CI) were also calculated. Both allele frequencies and genotype distributions did not significantly differ between UC, CD patients and the health control group.

Cases and Controls	Total numbers	Allele		Genotype			C vs T		CC vs. TT	
		C	T	CC	CT	TT	OR (95%CI)	P value	OR (95%CI)	P value
CD	186	181	191	45	91	50	0.95	>0.05	0.88	>0.05
CU	85	48.6%	51.4%	24.2%	48.9%	26.9%	0.72-1.27	>0.05	0.48-1.58	>0.05
		76	94	14	48	23	1.12	>0.05	1.30	
Controls	195	44.7%	55.3%	16.5%	56.5%	27.0%	0.78-1.6		0.59-2.41	
		185	205	81	82	32				
		47.4%	52.6%	41.5%	42%	16.5%				

Conclusions. These data enlarge the list of studies where no implication of C3435T *MDR1* polymorphism in UC or CD was detectable. These different results may be attributed to ethnic diversities of the studied populations and suggest that C3435T might not be the only polymorphism in *MDR1* gene implicated in the susceptibility to UC or CD.

P1097. GAS6 polymorphisms in patients with ischemic or hemorrhagic stroke

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Growth arrest-specific 6 gene product (GAS6) is a tyrosin 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 confirm these results with the analysis of 8 GAS6 SNPs in a larger population of 588 stroke patients (407 ischemic and 181 hemorrhagic) and in 127 healthy controls.

The genotypes for the 8 GAS6 polymorphisms were determined either by PCR and restriction analysis or by real time PCR and fluorescent probe hybridisation in a LightCycler.

Results: The prevalence of the AA genotype of GAS6 c.834+7 G>A was 12.2%*, 10.4%* and 18.2%, respectively in patients with ischemic stroke, hemorrhagic stroke and in controls (*chi-square, p<0.05 of AA vs non-AA compared with controls). After adjustment for age, gender, current smoking, hypertension, diabetes and hypercholesterolemia, the AA genotype was not independently associated with stroke (OR

0.74, 95% CI 0.39-1.42). The genotype distribution of the other 7 GAS6 polymorphisms (c.280+170C>G, c.712+26G>A, c.713-155T>C, c.1263G>C, c.1332C>T, c.1478-94C>G and c.1869T>C) did not differ between patients and controls.

Conclusions: The AA genotype of the GAS6 c.834+7 G>A SNP is less prevalent in patients with ischemic or hemorrhagic stroke than in healthy controls but this association is not independent from other vascular risk factors. The rest of GAS6 polymorphisms analysed are not associated with ischemic or hemorrhagic stroke.

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P1098. Linkage to rheumatoid factor seropositive rheumatoid arthritis confirms the involvement of the protein tyrosine phosphatase 22 gene

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Objective. The protein tyrosine phosphatase-22 (*PTPN22*) gene encodes for the lymphoid tyrosine phosphatase (LYP), involved in the negative regulation of early T cell activation. Recently, an association has been reported between *PTPN22*-620W functional allele and rheumatoid factor positive (RF⁺) rheumatoid arthritis (RA) as well as other autoimmune diseases. However, the linkage proof required to confirm a new genetic factor could not be definitely produced by an affected sib-pair (ASP) analysis. Our aim was to test this allele for linkage to RF⁺ RA with the transmission disequilibrium test (TDT).

Methods. DNA from the French Caucasian population was available for 2 sets of 100 families with one RA patient and both parents, and for 88 RA index cases from RA ASP families. Genotyping was done by PCR-RFLP. The analysis was performed using the TDT, the genotype relative risk (GRR) and a ASP-based tests.

Results. The TDT of the *PTPN22* 620W allele showed linkage and association for RF⁺ RA (61% transmission, $P = 0.037$). The GRR showed the risk allele in 34% of RF⁺ RA patients and in 24% of controls ($P = 0.047$, OR 1.69, 95%CI [1.03-2.78]). The ASP investigation showed no enriched risk allele in RA multiplex families, leading to a major lack of power of ASP analysis.

Conclusion. This study is the first to show clear linkage of *PTPN22* to RF⁺ RA, providing compelling evidence for *PTPN22* as a new RA, gene.

P1099. Hormone receptor variants and premature ovarian failure

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Premature Ovarian Failure (POF) is the occurrence of menopause before age 40, and affects approximately 1% of women. Variants in hormone receptor genes may affect hormone function and contribute to infertility and an earlier age at menopause. We therefore examined the presence of polymorphisms in hormone receptor genes including androgen receptor (*AR*) and estrogen receptor α (*ER α*) in POF patients and control women ascertained in Western Canada. The *AR* gene contains a translated, highly polymorphic CAG repeat that has been inversely correlated with receptor activity. Long *AR* repeats (≥ 24 CAG repeats) were found in 38 of 106 (36%) POF patient alleles and 162 of 588 (28%) control alleles ($p=0.08$). Since the *AR* gene is on the X chromosome, expression of alleles may be affected by X chromosome inactivation (XCI) skewing. We therefore assessed *AR* repeat size weighted by XCI ratio in a subset of patients and controls. XCI weighted *AR* repeat sizes ≥ 24 occurred in 15 of 53 (28%) POF patients and only 13 of 97 controls (13%) ($p=0.03$). A TA repeat in the *ER α* gene promoter was also examined and long repeats (≥ 18 TA repeats) were significantly more common in the POF patient population than in controls ($p=0.002$) (60/104 (58%) POF alleles vs. 84/214 (39%) control alleles). Assessment of polymorphisms in the estrogen receptor β and the FSH receptor genes are currently underway. Although we cannot exclude a confounding effect of ethnicity, these results suggest a role for functional hormone receptor variants in POF pathogenesis.

P1100. Molecular analysis of the Fragile X mental retardation syndrome in Volga-Ural region of Russia.

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Fragile X syndrome is most common inherited cause of mental retardation (MR). For the first time we have realized a study using molecular screening to estimate the prevalence of fragile X syndrome among mentally retarded children of Bashkortostan. Molecular screening was carried out using methylation-sensitive polymerase chain reaction (PCR) to detect CpG methylation. We used DNA samples from male patients at the age of 3 to 18 years with non-specific MR (n=214). We found that 4.2% (n=9) of mentally retarded male screened by DNA analysis had the fragile X full mutation. This result is very close to the prevalence of the fragile X syndrome in Caucasian MR population, where it accounts for 2.6 to 8.7% among moderate to severely retarded males.

In order to investigate the origin of the fragile X mutation, we assessed the size of the microsatellite markers DXS998, DXS548 and FRAXAC1 in FRAXA patients and control groups. We found a different distribution of alleles between fragile X patients and controls for loci DXS998, FRAXAC1, but not detected apparent linkage disequilibrium for DXS548 locus. The frequencies of DXS998/FRAXAC1 haplotypes 117/152 bp (3-4) and 113/156 (1-2) were significantly greater in affected males in comparison with healthy donors.

The data of this study revealed significant differences in the distribution of DXS998/FRAXAC1 haplotypes between fragile X patients and controls.

P1101. Selection of putative functional coding SNP for direct genetic association studies of complex traits

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Genetic association studies of complex disease can be: indirect, when surrogate markers in linkage disequilibrium (LD) with the disease allele are tested for trait association; or direct, in which a list of putatively functional SNPs, non-synonymous coding variants (nsSNPs), are tested for their disease relevance directly. An indirect genome scan would probably need from 120,000 to 1 million SNPs - an enormous genotyping cost and a problem of statistical inference. A direct genome scan may require typing only tens of thousands common nsSNPs. The feasibility of such studies requires that most of the common variants influencing the susceptibility to complex disease are available for genotyping. About 40,000 candidate nsSNPs are available on public databases and an additional 30,000 novel nsSNPs were discovered by resequencing the exons of 23,363 human genes. Combined, these datasets provide a comprehensive resource of nsSNPs making feasible the execution of candidate gene/region and whole genome direct association studies. We designed genotyping assays for these nsSNPs as TaqMan® SNP Genotyping Assays and for the SNPlex™ Genotyping System, a high throughput implementation of the oligonucleotide ligation assay. We prioritized a subset of 28,709 nsSNPs, including over 9,000 novel SNPs, based on their measured or expected heterozygosity in populations of European and African descent. This list represents the common nsSNPs in the genome and an analysis of the gene classes overrepresented therein meets our expectations of genes that can tolerate slightly deleterious mutations. These assay resources are available to researchers through our SNPbrowser™ Software and our web site.

P1102. Association of ADH1B, ADH7, CYP2E1 gene polymorphism with alcohol dependence in Russian populations of Siberia

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The allele and genotype distribution of two alcohol dehydrogenase genes ADH1B (exon 3 polymorphism A/G (47His)), ADH7 (intron 5

polymorphism G/C) and cytochrome P450 2E1 gene (CYP2E1; 5'-flanking region polymorphism G/C) were examined in two urban (Seversk, n=124 and Tomsk, n=112) and one rural (Kargala, n=96) Russian populations. No interpopulation or sex difference in allele frequencies was revealed. The genotype frequencies obeyed the Hardy-Weinberg equilibrium and the alleles were in linkage equilibrium or gametic equilibrium in the total sample. The frequencies of the derived alleles at ADH1B (*G (+Msl I) allele) and CYP2E1 (*C (+Pst I) allele) were low (5.87% and 2.56%, accordingly). ADH7 gene polymorphism show the high level of genetic variability; the frequency of the "mutant" ADH7*C (-Sty I) allele was 45.48%. Similar allele frequencies of these genes were revealed in most Caucasoid populations. The alcohol dehydrogenase gene polymorphisms were significantly associated with an increased risk of comorbidity of alcoholism and tuberculosis (n=83) in Tomsk population. The frequencies of the derived ADH1B*G, ADH7*C and CYP2E1*C allele were 1.81%, 54.82% and 1.81% in patients accordingly. In individuals with ADH1B*A allele odds ratio for comorbidity of alcoholism and tuberculosis is 3.39 (95% CI 0.99 - 13.93; P=0.052) and in individuals with ADH7*G allele - 0.69 (95% CI 0.48 - 0.98; P=0.031). The ADH1B*G (*47His) allele encodes enzyme with higher activity, likely causing protection against alcoholism. Possibly the association with ADH7*G allele are explained by linkage disequilibrium with other functionally significant loci or by stochastic reasons.

P1103. Mutation detection in a large cohort of patients with Common Variable Immunodeficiency using Multiplex Capillary Heteroduplex Analysis (MCHA)

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Common Variable Immunodeficiency (CVID), one of the most common primary immunodeficiency syndromes, is a heterogeneous group of disorders characterized by hypogammaglobulinemia and specific antibody deficiency resulting in recurrent infective and inflammatory problems. Although most cases of CVID are sporadic, about 20-25% are familial, displaying autosomal dominant and autosomal recessive modes of inheritance. CVID patients have T cell defects as well as defects in terminal B cell differentiation. Although the genetic defect is unknown, a number of genes are now emerging which may be good candidates to screen to identify molecular defects in CVID. Detection of unknown mutations in a large number of genes and a large number of samples through DNA sequencing is an expensive and time-consuming process. In our cohort of CVID patients, we adopted Multiplex Capillary Heteroduplex Analysis (MCHA) as a method to rapidly identify DNA fragments containing mutations, the exact nature of which may subsequently be determined by DNA sequencing. MCHA is a simple and rapid assay performed on a 96-capillary sequencer, the MegaBACE 1000, and has the ability to detect point mutations with high sensitivity. High throughput is achieved by multiplexing compatible fragments in the same capillary and by analysing 96 samples in 40 minutes. The overall mutation detection rate is currently over 95%, confirming the usefulness of this particular method for high throughput mutation detection.

P1104. DGGE analysis of several genes in patients with (idiopathic) Dilated Cardiomyopathy referred to the combined cardiogenetics clinic

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Dilated cardiomyopathy (DCM) is a heart muscle disease characterised by left ventricular dilatation with impaired contraction of the left ventricle. Dilated cardiomyopathy is also associated with limb girdle muscular dystrophy 1B (LGMD1B) and Emery-Dreifuss muscular dystrophy (EDMD). Idiopathic DCM is familial in up to 30% of cases. Mutations in different genes have been identified as disease associated, among others in the gene encoding lamin A/C (LMNA), delta-sarcoglycan (SGCD), troponin I (TNNI3), beta-myosin heavy chain (MYH7), desmin,

cardiac actin, dystrophin and tafazzin. The most common mode of inheritance in families with DCM is autosomal dominant and the most commonly mutated gene so far is *LMNA* with a mutation frequency of 5-10% of all DCM cases.

In recent years we collected material from more than 100 patients referred to our combined cardiogenetics outpatients clinic. To identify the genes involved in (familial) DCM or DCM associated with muscular disease in our population we developed an efficient screening method, based on denaturing gradient gel electrophoresis (DGGE) and sequence analysis, for the *LMNA*, *SGCD*, *MYH7* and *TTN3* genes. Up to now eleven different mutations have been found in *LMNA*, two in *TTN3*, and none in *SGCD* and *MYH7*, of which five had not been described before. The most remarkable finding was the identification of an *LMNA* mutation in a family with pure cardiac conduction disease (CCD), A225X, thereby expanding the clinical spectrum of laminopathies, although CCD can precede ventricular dilatation up to 20 years.

P1105. Familial steroid responsive nephrotic syndrome: search for a candidate gene

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The etiology for Steroid-responsive idiopathic nephrotic syndrome (SSINS), the common form of childhood nephrosis is unknown. We identified an extended Bedouin family with a high rate of consanguinity, suitable for linkage analysis (LOD score of 3.5 for a model marker at 0cM distance for recessive inheritance). Its 10 affected individuals' clinical presentation and steroid response are similar to the non-familial SSINS. Retrospective analysis of all children with INS treated by our institution in the past 15 years revealed another 5 non-related Bedouin families with 2-3 first-degree cousins affected with SSINS in each. The overall familial SSINS rate among the Bedouin population (excluding the index family) is 26%, contrary to 4.7% among Jewish.

DNA was extracted from 8 affected individuals in the index family and 16 of their non affected siblings and parents. Genome wide linkage analysis with a panel of 387 supplemented by 70 polymorphic markers, aimed to limit intervals to ~10cM and verify markers showing allele homozigotization, failed to identify linkage based on autosomal recessive and X-linked recessive models of inheritance. In addition, linkage was excluded to 8 candidate genes associated with NS by the use of adjacent polymorphic markers. In summary: Although the Bedouin population presents an increased familial tendency for SSINS (milder diseases), this disease is probably not caused by the homozigotization of one major susceptibility gene since autosomal recessive and X-linked recessive inheritances were ruled out by genetic analysis of a statistically significant family. Autosomal dominant (with partial penetrance) and multifactorial inheritances cannot still be ruled out

P1106. Association of the serotonin receptor 5HT2C with eating disorders and related psychopathological traits

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Several lines of investigation support a serotoninergic participation in eating behaviour and body weight regulation and suggest its involvement in the aetiology of eating disorders (ED) and some related psychopathological traits. To test this hypothesis we have analyzed the -995G>A, -759C>T, -697G>C and Cys23Ser SNPs within the serotonin receptor 5HT2C gene by a population-based association study in a total sample of 151 ED patients and 116 sex-matched unrelated controls. We also analyzed the potential involvement of the 5HT2C gene in different psychiatric symptoms measured by the Symptom Checklist 90-revised (SCL90-R) questionnaire. The case-control study showed that the -995G/-759C/-697C/Ser23 haplotype was associated to the purging ED categories of and binge-eating/purging anorexia and bulimia nervosa ($P = 0.01$). We also observed that bulimic patients carrying the -995A/-759T/-697C/Cys23 5HT2C haplotype showed increased symptomatology for seven of the nine subscales of the SCL90R questionnaire. These include somatization

($P = 0.029$), obsessive-compulsiveness ($P = 0.021$), depression ($P = 0.032$), anxiety ($P = 0.004$), hostility ($P = 0.028$), phobic anxiety ($P = 0.029$) and paranoid ideation ($P = 0.008$). The results presented here suggest that the 5HT2C gene may participate in the physiopathology of ED, not only through its direct effect on eating behaviour, but also on the anxiety and depressive traits associated to anorexia and bulimia nervosa.

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P1107. Mutational analysis of the *PROP1* gene in Polish patients with combined pituitary hormone deficiency (CPHD)

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Genetically determined congenital form of the combined pituitary hormone deficiency (CPHD) occurs in about 10% of patients with impaired production and secretion of anterior pituitary hormones. *PT1* (*POU1F1*), *HESX1*, *PITX1*, *PITX2*, *LHX3*, *LHX4* and *PROP1* genes are involved in ontogenesis of anterior pituitary gland. The *PROP1* gene, located on chromosome 5q35, consists of three exons and spans 4 kb. Gene product, a tissue specific paired-like homeodomain transcription factor consists of 226 amino acids. Mutations in *PROP1* gene appear to be one of the most common causes of familial CPHD and may account for up to 50% of all cases. We analyzed *PROP1* mutations in a group of 35 Polish patients from 32 families with CPHD and gene defects were found in fifteen (42.9%) patients. Analysis revealed three different mutations: c.150delA, c.301_302delAG and novel transition c.334C>T causing p.Arg112X substitution. Six patients were found to be homozygous for c.150delA and eight such mutations were in compound heterozygosity with c.301_302delAG mutation (6 cases) or with substitution c.334C>T (two brothers). One patient was found to be heterozygous for mutation c.150delA but compound mutation at other allele was not found. The most frequent mutation - deletion c.150delA (72.4% of all found mutations) is rare in other populations. Genotyping of marker D5S408 linked to the *PROP1* gene suggests that c.150delA in Polish patients is not a result of single common founder mutation.

P1108. Studies of chimerism in twins

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It is already known that in vitro fertilization is associated with an increase in embryo splitting and monozygotic twinning. It may be also associated with an increase risk of embryonic fusion before implantation. Zygosity studies on DNA extracted from peripheral blood of 87 twins conceived by in vitro fertilization, born in Clinics of University of Medical Sciences, were undertaken. Analysis of restriction fragments length polymorphism (RFLP) detected by hybridization with molecular probes and detection of polymorphic minisatellite and microsatellite DNA sequences (STR) by PCR was performed. Nine genetic markers D1S7, D1S80, TPOX, D7S21, TH01, D12S11, PLA2A1, VWA and CYAR04 were analyzed. Quantitative studies were performed using radioactivity scanning of RFLP blots hybridized with molecular probes. The results of analysis show that in four cases among 173 twins blood chimerism may be considered, which suggests that the influence of in vitro fertilization on early embryonic development requires further investigation.

P1109. Analysis of genetic variants of *NOD2/CARD15* and *DLG5* gene in a Polish family with daughter suffering from Crohn's disease

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Crohn's disease (CD) belongs to inflammatory bowel diseases (IBD) with growing frequency, especially a form involving the colon. Molecular background is still unknown, although recent data suggest that

mutations in *NOD2/CARD15* gene and genetic variants in *DLG5* gene are associated with the disease. In studied case the female patient's development was normal until the age of 19 years when symptoms of CD appeared. Main complains were: diarrhea without the presence of RBC in bowel movements, cramp abdominal pain, nausea, vomiting, lost of weight. On examination the painful tumor in abdominal right lower quadrant was still palpable. The abnormalities in laboratory tests and other parameters were detected. Ultrasound examination showed thickened wall of distal part of ileum and colonoscopy showed narrowed lumen in ascending colon with the presence of ulcerations and pseudo polyps. CD was confirmed histopathologically.

Molecular analysis of *NOD2/CARD15* and *DLG5* gene was performed on patient's DNA and family members without any symptoms of disease, using screening methods and DNA sequencing. Analysis of patient showed homozygous presence of two frequent sequence variants: 802C>T (Pro268Ser) and 3020insC (1007fs). Variant 802C>T in both alleles appeared in brother and father of affected girl as well, whereas mother was heterozygous. The 3020insC was homozygous in patient sample only, whereas the other family members were heterozygous. The first conclusion is that complete disruption of *NOD2*-signaling pathway may be crucial in development of the disease, but the other factors, such as the others susceptibility genes as *DLG5* or co-existing infection could also play important role.

P110. Glucokinase mutations and low triglycerides

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Mutations in the glucokinase gene (GCK) lead to an impaired sensing of blood glucose by the pancreatic β -cell causing an autosomal dominant form of type 2 diabetes, Maturity-Onset Diabetes of the Young type 2 (MODY2). Usually, MODY2 patients present increased fasting blood glucose levels (6-12 mM) from an early age on which should significantly increase their risk for diabetic complications. Whereas other forms of diabetes lead to macrovascular complications, MODY2 patients rarely develop those problems.

In a family with autosomal dominant diabetes we identified a novel C to A substitution (A232D) in the GCK gene in all affected individuals. Mutations around codon 232 cause a very low glucokinase activity and suggest a similar effect of A232D.

Elevated HbA_{1c} levels should correspond with elevated triglycerides since they indicate a disturbed metabolic control. However, despite off an elevated HbA_{1c} affected subjects showed even lower fasting triglycerides than the unaffected persons (affected/unaffected (n=7/13): Triglycerides 0.65/1.56±0.31/0.82 mM (P=0.025), HbA_{1c} 6.63/5.40±0.26/0.28% (P=0.0000028)). We made the same observation in another MODY2 family.

GCK mutations should lead to lower triglycerides, even within the normal range (0.35-2.30 mM), since they impair glycolysis which is responsible for delivering glyceraldehyde-3-phosphate as the later glycerol backbone of triglycerides. Transgenic mice overexpressing GCK develop hypertriglyceridemia, therefore indicating a link between glucokinase activity and triglyceride regulation.

In conclusion, an impaired GCK activity could cause particularly low triglycerides. The contribution of low triglyceride levels to lesser macrovascular complications in MODY2 patients remains a matter of future investigation.

P111. easyLINKAGE Plus - Automated single-/multipoint linkage analysis for microsatellites and large scale SNP data in an user-friendly Windows environment

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Most of linkage analysis software was traditionally developed for UNIX environments restricting calculations to experienced users. Input files had to be generated in a time-consuming process and graphical output

capabilities were limited.

We have generated the program easyLINKAGE that combines automated setup and performance of linkage analyses and simulation under an easy to handle graphical user interface for Microsoft Windows 2000/XP. The program can analyze microsatellite as well as SNP data (Affymetrix 10k chip). easyLINKAGE supports single-point linkage analyses (FastLink, SuperLink, SPLink), multi-point linkage analyses (GeneHunter-Plus, Allegro), and single-point simulation studies (SLink). In particular, Allegro can now be used for SNP chip data because it is not limited by marker numbers. easyLINKAGE Plus tests for Mendelian errors prior subsequent linkage analyses based on PedCheck. The program uses predefined marker databases (Marshfield, decode, LDB, SLM1). The user can choose between five different allele frequency algorithms plus additional allele frequency reference values for Asian, Caucasian, African-American populations for SNP chip projects (data kindly provided by Affymetrix Inc.). easyLINKAGE provides genome-wide as well as chromosomal graphical plots of LOD scores, NPL scores, P values, and many other parameters. The program generates input files for pedigree/haplotype drawing software such as HaploPainter.

There is currently no other program available that offers the comfort and versatility of easyLINKAGE at a comparable level. The design enables the use of the program for a wide audience.

P112. Attention-deficit/hyperactivity disorder (ADHD) association with the DAT1 core promoter -67 T-allele

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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 100 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 15.5%; AT 71.8%; TT 12.6% vs. the genotype frequencies in the control group: AA 49%; AT 41.8%; TT 9.2% [$\chi^2=31.11$, df = 2, OR = 2.15 (95% CI 1.34-3.47, $p \leq 0.0001$]. The T-allele of the -67A/T polymorphism revealed a ~1.6-fold excess in the patients group comparing with the controls ($\chi^2=18.45$, df = 1, $p \leq 0.001$). 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.

P113. Genetic predisposition for cardiac hypertrophy of different origin

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Cardiac hypertrophy (CH) is a leading predictor of heart failure, it considered being adaptive response to a variety of stimulus (excessive hemodynamic burden, endocrine abnormalities, mutations in sarcomeric protein genes etc.). Nevertheless, common genetic mechanisms and common genes may exist for etiologically distinct CH forms.

We studied three groups of patients with diverse initial events for CH: 136 - with essential hypertension (64% had left ventricular hypertrophy (LVH)), 95 - with arterial hypertension, combined with diabetes mellitus type 2 (53% with LVH) and 32 - with hypertrophic cardiomyopathy. Analysis of 15 polymorphisms in 9 candidate genes (ACE, AGTR1, GNB3, NOS3, TNF, PPP3CA, GATA4, MYH7, MYBPC3) revealed that the majority of genes (5 of 8) where associations with echocardiographic parameters were detected are common for two or three CH forms,

although individual effects varied considerably. Some distinctions in genetic basis of different CH forms were found: while in essential hypertension *ACE* (A-240T and A2350G) and *AGTR1* (A1166C) genotypes accounted for 6.0-9.0% of variability of left ventricular mass index (LVMI), no substantial effect of renin-angiotensin system genes was found in hypertensive patients with DM2. On the contrary, polymorphisms of *NOS3* (C774T and G894T) accounted for 7.0-8.0% of LVMI variability in DM2 group, while for essential hypertension were no associations with LVMI. In hypertrophic cardiomyopathy expression of LVH depended on contractile protein gene polymorphisms (T1575C of *MYH7* accounted for 14% of variance). The comprehensive approach to CH genetic analysis has revealed features, which can remain hidden when single forms of CH are studied.

P1114. Molecular genetic study for the prenatal diagnosis of hemophilia B by linkage analysis

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Linkage analysis is a very useful method for prenatal diagnosis or carrier status evaluation of Hemophilia B, especially when a mutation was not identified in a family. Eleven polymorphic markers were evaluated in Korean populations. We analyzed nine intragenic markers within the factor IX gene and two extragenic microsatellite markers in 100 healthy Korean women (200 X-chromosomes). *Sall*, *Msel*, *Nrul*, *Ddel*, *Xmn1*, *TaqI*, *Mnl I* and *HhaI* polymorphisms were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, DXS 102 and DXS 1232 microsatellite markers by DNA fragment analyses with GeneticAnalyzer, and T/C nucleotide variation at nucleotide 32770 in the poly-A region by fluorescence melting curve analysis with real-time PCR machine. There was no polymorphism of *Ddel*, *Xmn1* and *TaqI* markers in Korean population. There was complete linkage disequilibrium between *Sall*(+) allele and *Mse*(-) allele, and vice versa. The expected heterozygosity of *Sall*, *Nrul*, *Mnl*, *HhaI* were estimated to be 45.8%, 24.8%, 8.3%, 32%, respectively. The repeated number of (AC)_n dinucleotide in DXS 102 and DXS 1232 ranged 13-20 and 21-30 repeats, respectively, and expected heterozygosities were 36.8% and 74.8% respectively. T/C Polymorphism at the poly-A region (nt32770) showed expected heterozygosity of 15%. With the 7 informative polymorphisms together, the expected heterozygosity rate was improved to 95.86%. Therefore the prenatal diagnosis and carrier detection in hemophilia B families can be achieved effectively and rapidly in Korean with these polymorphisms.

P1115. A gene causing cataract and hypogonadism maps to the long arm of chromosome 2

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We have encountered an extended inbred Arab Muslim family in which four members suffer from congenital cataract, developmental delay and hypogonadism. Laboratory tests indicated high levels of FSH and LH and low levels of testosterone, findings indicative of primary hypogonadism. The disease segregates in an autosomal recessive mode and is consistent with previous descriptions of Martsolf's syndrome. A genome wide search performed on DNA samples from 15 family members, revealed initial linkage to chromosome 2q. A maximal lod score of 3.51 was obtained with the marker D2S1334 at θ=0.00. Fine mapping with self constructed polymorphic CA repeats confined the disease to 1 Mb interval between the genomic clones AC012450 and AC112255. Sequencing of seven candidate genes from the interval, expressed in the eye, brain and gonads failed to disclose disease-associated mutations. Additional genes are currently being sequenced.

P1116. Use of the NanoChip platform for genotyping TNFα gene SNPs likely to be involved in the pathogenesis of COPD.

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Chronic obstructive pulmonary disease (COPD) is associated with abnormal inflammatory response of the lungs to noxious particles or gases. Tumor Necrosis Factor -α (TNF-α) gene polymorphisms are suspected to play an important role in disease susceptibility. TNF-α gene SNPs:-1031T>C, -863C>A, -857C>T, -308G>A, and -238G>A, were examined in 95 Greek COPD patients diagnosed according to GOLD guidelines (FEV1/FVC < 70% and FEV1<80% of predicted values) and compared to 96 Greek control subjects without evidence of COPD or other lung disease. Biotinylated primers specific for the promoter sequence of the TNF-α gene were used for PCR. SNPs were genotyped using the NanoChip™ Molecular Biology Workstation (Nanogen www.nanogen.com) using the amplicon down format. To reduce the cost of the assay we developed a universal reporting system 3' labeled with fluorescent dyes Alexa555 (wild-type) and Alexa647 (mutant). The target sequences on the 100 electronically addressable sites were hybridized to specific oligonucleotide reporters in conditions that allow discrimination between mutant, heterozygous and wild-type samples. For the 5 SNPs studied no statistical significant difference was found between COPD patients and controls.

SNP position	genotype	COPD Genotype frequencies	CONTROLS Genotype frequencies	Statistical analysis
-1031 T/C	TT	0.6667	0.6042	P=0.2
	TC	0.3333	0.3646	
	CC	-	0.0312	
-863 C/A	CC	0.7143	0.625	P=0.14
	CA	0.2857	0.3438	
	AA	-	0.0312	
-857 C/T	CC	0.5555	0.5053	P=0.75
	CT	0.3889	0.4210	
	TT	0.0556	0.0737	
-308 G/A	GG	0.8737	0.8260	P=0.33
	GA	0.1158	0.1740	
	AA	0.0105	-	
-238 G/A	GG	0.9551	0.9674	P=0.66
	GA	0.0449	0.0326	
	AA	-	-	

P1117. Confirmation of psoriasis susceptibility genes on chromosomes 6p21 and 20p12 by linkage and family-based association study in French families

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Psoriasis is a common skin disorder affecting 1 to 4 % of the population worldwide. The trait is widely regarded to be multifactorial in origin including gene-gene and gene-environnement interactions. Genetic and allelic heterogeneity, complex inheritance, and low penetrance of susceptibility alleles substantially complicate both study design and interpretation of results. Genome-wide scans for disease susceptibility have repeatedly demonstrated the existence of a major locus, PSORS1, which encompasses 3 genes (HLA-C, HCR and CDSN) carrying psoriasis-associated SNPs. Subsequently, 20 additional loci have been suggested in the literature. With the aim of elucidating the genetics of psoriasis, a study was initiated at Généthon in 1996, which led to the identification of 3800 families through a national media campaign. From this collection, 46 large families with over 8 affected individuals and apparently autosomal dominant inheritance were pre-selected to carry out a genome-wide search. Our results confirm previous findings of linkage to PSORS1 and support the presence of a susceptibility locus on proximal chromosome 20p. Genotyping for HLA-C alleles and SNPs within genes HCR and CDSN demonstrates association with PSORS1 alleles HLA-Cw6, CDSN 971T (p=0.0001), HCR 325T (p= 0.0003), HCR 1723T (p=0.0001) and HCR 2327G (p=0.0003). Using a dense SNP map to narrow the area of linkage on chromosome 20p, we also identified a risk allele for the disease in a new candidate gene. Further studies are needed to evaluate the proportion of families linked to 6p21 and to 20p12, and to determine whether the 2 loci interact.

P1118. A new player in the field of celiac disease? Results from fine-mapping the CELIAC4 region

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Celiac disease (CD) is a complex genetic disorder. Besides the environmental factor gluten and the HLA-DQ2 and 8 proteins, other unknown genetic factors are involved. Several genome-wide screens have been performed to locate the regions with genes involved in CD. In the Dutch population this has led to the discovery of two susceptibility regions, 6q21-22 and 19p13 (CELIAC4). The region on 19p13 is only limited to 3.5 Mb, but due to its high density of genes, it still contains 92 candidate genes. We set out to fine-map this region with microsatellite markers and single nucleotide polymorphisms (SNPs) to search for association between genes and CD. We started with a cohort of 216 cases and 216 controls and expanded this to 311 cases and 540 controls. Association testing using microsatellite markers has revealed a small region of interest of around 450 kb. Further fine-mapping with SNP shows association in a 150 kb region, encompassing a limited number of genes. Adding more SNPs led to the discovery of MYO9B as the gene on 19p13 most strongly associated to CD. This gene, which is a single-headed motor myosin, shows association in its 3' part. This part of the gene contains the most interesting domains, differentiating the role of this myosin from the other family members. The Rho-Gap and Dag-Pe domains indicate that this gene is involved in signal transduction. We are now elucidating the specific role of this gene in signal transduction and trying to incorporate it in our models for CD.

P1119. ABCA1 gene R1587K polymorphism and coronary artery disease in CAD probands and sibs from Russia

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Coronary artery disease (CAD) is multifactorial disease, and its progression is known to be influenced by different risk factors. Plasma lipoprotein disturbances are among the most common biochemical abnormalities observed in patients with CAD. Among the lipoproteins, HDL-C level are strongly inversely correlated with CAD. Recently, the ABCA1 protein was identified as an mediator of cholesterol efflux. A number of common polymorphisms have been reported in the coding and promoter regions of ABCA1 gene. We have analyzed single-nucleotide polymorphism R1587K in a group of patients with angiography proven coronary artery disease and their sibs from Russian population. To assess the influence of R1587K polymorphism on the clinical and biochemical characteristics in proband and sibs groups non parametric gamma correlation analysis was performed. For probands with CAD the R1587K polymorphism does not influence the main CAD clinical and degree of an coronary arteries atherosclerotic lesion. It is not revealed also effects of this polymorphism on a plasma lipid spectrum. At the same time for CAD patient sibs the strong and very significant correlation between a R1587K CAD, myocardial infarction and angina is found. Thus the increased risk of CAD and its main manifestations is associated with R1587 allele - relative risk of CAD development for genotypes RR and RK carriers is 4,88 (95 % CI 4,14-7,62). This association of a R1587K polymorphism with risk of CAD development in sibs group is not linked with its effect on a plasma lipid levels.

P1120. Two p53 polymorphisms modify cancer risk in BRCA2 mutation carriers

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Germ-line mutations in the BRCA genes confer a high life-time risk to develop breast and other cancers. Although these mutations are considered as highly penetrant, estimates of risks actually may vary from 36% to 70% to develop breast cancer at age 70, depending on the family ascertainment and the population studied. Other genetic and/or environmental factors, called "modifiers" are supposed to be

the cause of these differences not only in the risk to develop breast cancer but also in the appearance of other associated tumours.

The aim of the present study was to investigate the role of the two p53 polymorphisms, 16bp insertion and Arg72Pro, as BRCA1/2 modifiers. For this purpose we investigated the possible association between the two polymorphisms and disease status in 424 BRCA1/BRCA2 mutation carriers belonging to 170 breast and/or ovarian cancer Spanish families. Genotype and haplotype analysis revealed that the presence of a specific haplotype carrying the wild-type allele for the 16bpins and the variant allele for the Arg72Pro (WT-72Pro haplotype) was associated with an earlier age of onset in BRCA2 mutation carriers. We found almost a three-fold increase risk to develop the first primary tumour (breast or ovarian) before 35 years of age considering all the carrier women OR: 2.77 (95%CI: 1.05-7.31 p=0.039). Considering index cases only, results were even more significant given that younger cases were over-represented (OR:5.29 CI95% 1.48-18.8 p=0.001). The finding of concordant and statistically significant results in both analysis allow us to conclude that these polymorphisms in p53 modify BRCA2 penetrance.

P1121. DIAPH2 is a susceptibility gene for Premature Ovarian Failure (POF)

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Premature Ovarian Failure (POF) is an heterogeneous genetic disorder characterized by lack of ovulation and elevated gonadotropin level before 40 years of age. POF has a frequency of about 1% and with the increase of women reproductive age it has become a relevant cause of infertility. The DIAPH2 gene in Xq22 is one of the two human homologues of the Drosophila dia gene, whose mutated alleles affect fruit fly fertility. DIAPH2 was interrupted by a balanced X/autosome translocation in a familial case of POF. We performed a case-control study on a panel of 255 Italian patients and 404 matched controls to identify allelic variants in DIAPH2 associated with the disorder. The haplotypic structure of the gene was constructed using a pair-wise maximum likelihood estimation method. Inside LD block 1, one SNP (L2) showed a significant excess of heterozygotes (X-square test, 1df, p=0.002) among patients, resulting in the definition of a risk-genotype (O.R.=1,67; 95%CI: 1,2-2,3). The excess of heterozygotes at the L2 locus may be the result of different genetic mechanisms. One possible explanation is that the risk-haplotype would be lethal in male embryos: accordingly, the number of females and males in the offspring of familial POF cases carrying the DIAPH2 risk-genotype demonstrated a significant excess of females (31 females/13 males; One-cell X-square p=0.007). Our data demonstrated that the DIAPH2 gene is involved in the pathogenesis of POF and that a functional variant in the LD block 1 may be responsible for ovarian dysfunction.

P1122. Association of Glutathion-S-transferase M1, T1 and P1 Gene Polymorphisms with Autoimmune Hepatitis (AIH)

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Autoimmune hepatitis (AIH) is a chronic inflammatory liver disorder of unknown etiology. GST (Glutathion-s-transferases) genes, enzymes metabolizing carcinogens, drug, and foreign compounds, may play a role in susceptibility to autoimmune liver disease and its severity. We determined the frequency of GSTM1, GSTT1 and GSTP1 polymorphisms in Iranian patients with AIH. METHODS: In a case-control study, we examined 64 AIH patients (49 female and 15 male), referred to RCGLD, and 100 healthy controls (70 female and 30 male), after obtaining an informed consent. The mean ages for cases and controls were 35.4 ± 13.4 and 32.1 ± 7.5 , respectively. After DNA extraction by salting out method, the genetic polymorphism analysis for GST M1 and the GST T1 genes was determined in a single assay using a multiplex PCR approach. Also, PCR-RFLP method was used for detection of GST P1 gene polymorphism. RESULTS: GSTM1 and GSTT1 null genotypes (deletions) were determined in 33(51.6%) and 15 (23.4%) patients with AIH, and 56(56%) and 22(22%) controls, respectively. Comparison of patients and controls relative to GSTM1

and GSTT1 genotypes revealed no significant difference between them. Regarding GSTP1 genotypes, 25(39.1%) heterozygotes, 7 (10.9%) homozygotes in the case group and 38(38%) heterozygotes, 14(14%) homozygotes in the control group were observed. The allele frequency of GSTP1 was 30.4 and 33 in patients and controls, respectively showing no significant variation among cases and controls. CONCLUSION: Our study dose not support any specific role for GST genotypes in AIH which may be because of the variation in ethnicity.

P1123. Investigation of common genetic factors in autoimmune diseases

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Different autoimmune diseases (AIDs) share part of their genetic background. T-cells are important mediators of AIDs, whereas cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and protein tyrosine phosphatase (PTPN22) are both negative regulators of T-cell activation and proliferation. To establish the association of both genes with AIDs, we tested SNPs in *CTLA4* and *PTPN22* in three independent groups of AID patients.

350 Type 1 Diabetes (T1D), 309 Coeliac Disease (CD) and 153 Rheumatoid Arthritis patients (RA) were tested for association in a case/control design with four SNPs in the *CTLA4* gene region (MH30*G/C; -1149*C/T; CT60*G/A; JO37_3*G/A) and one coding SNP 1858C>T in the *PTPN22* gene. In addition, 218 T1D families were tested by the transmission disequilibrium test (TDT).

The T1D group showed strong association to *PTPN22* 1858C>T, both in the case/control design and by TDT ($p=2 \times 10^{-7}$ and $p=9 \times 10^{-9}$, respectively). The T1D group also showed association to the *CTLA4* gene ($p=0.003$ and $p=0.02$ for the most associated SNP in the case/controls and TDT, respectively). We observed an association with *PTPN22* in the RA group ($p=0.003$) but found no significant association to *CTLA4*, although there was a trend towards an increase in the T1D-susceptible haplotype. No association between *CTLA4* and *PTPN22* was observed in the CD patients. We are now investigating the interaction of these two genes.

We conclude the *CTLA4* and *PTPN22* genes are associated with an increased susceptibility to some AIDs. Investigating the co-association of various genes and using stratification protocols might provide clues to understanding the genetic basis of AIDs.

P1124. New candidate members of the HLA 8.1 ancestral haplotype (8.1 AH): RAGE -429C and HSP70-2G

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Introduction: The 8.1. AH is common in the Caucasian population. This haplotype consists of the HLA-B8, HLA-DR3(17) and HLA-DQ2 alleles, and in the MHC III region contains the TNF- α -308A (TNF2), monomodular short RCCX module (MonoS) with a single C4B gene but no C4A gene. 8.1AH is found to be associated with many autoimmune diseases such as type 1 diabetes mellitus (T1D).

Methods and materials: 93 healthy Hungarians (HH), 97 healthy female Caucasians from Ohio (HFO) and 91 healthy Icelandic (HI) subjects were genotyped for TNF- α -308, HSP70 -2, and RAGE -429 polymorphisms by PCR-RFLP, and for C4A and C4B polymorphisms in the RCCX module (Blanchong et al, JEM, 2000), 196 Hungarian individuals from families affected with T1D were genotyped for the above mentioned regions, and for HLA-DR and DQ alleles by SSP-PCR. Linkage disequilibrium (LD) coefficients were calculated by the Arlequin software.

Results:

Combination of alleles		HH	HFO	HI
TNF2	HSP70-2G	$p=7.58 \times 10^{-4}$	$p=5.32 \times 10^{-4}$	$p=8.81 \times 10^{-3}$
	RAGE -429C	$p=8.02 \times 10^{-5}$	$p=2.39 \times 10^{-5}$	$p=3.82 \times 10^{-5}$
C4A*Q0 (C4A<C4B)	HSP70-2G	$p=1.13 \times 10^{-4}$	$p=0.285$	$p=4.87 \times 10^{-4}$
	RAGE -429C	$p=5.63 \times 10^{-6}$	$p=0.00022$	$p=0.00086$
MonoS-C4B	HSP70-2G	$p=6.82 \times 10^{-6}$	$p=6.08 \times 10^{-5}$	$p=0.0767302$
	RAGE -429C	$p=7.17 \times 10^{-15}$	$p=3.13 \times 10^{-6}$	$p=1.81 \times 10^{-6}$

Hungarian families affected with T1D

	HLA-DQ2	HLA-DR17	TNF2	MonoS
HSP70-2G	$p=2.82 \times 10^{-10}$	$p=1.57 \times 10^{-9}$	$p=3.7 \times 10^{-9}$	$p=1.57 \times 10^{-5}$
RAGE-429C	$p=6.93 \times 10^{-11}$	$p=1.07 \times 10^{-19}$	$p=1.69 \times 10^{-15}$	$p=5.36 \times 10^{-6}$

Discussion: Our results indicate that there is strong LD between the RAGE -429 C and HSP70 -2G alleles with alleles known to be members of the 8.1 AH. Therefore it can be assumed that RAGE -429 C and HSP70 -2 G alleles are new candidate members of the 8.1 AH.

P1125. Absence of association between the IVS6+79G/A polymorphism in PROZ and stroke

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Plasma levels of Protein Z (PZ), a vitamin K dependent protein with both procoagulant and anticoagulant properties, show a wide interindividual variation and different case-control studies have revealed contradictory results on the association between PZ plasma levels and cerebral ischemia. Recently, an association between the IVS6+79G/A polymorphism in the PZ gene (PROZ), PZ plasma levels and cerebral ischemia in young individuals has been described. The proposal of our study was to confirm these results in our population of patients with stroke.

The IVS6+79G/A polymorphism of PROZ has been analyzed in 549 patients with ischemic (n=387) or hemorrhagic (n=162) stroke and in 127 healthy controls, with an average age of 66, 72 and 64 years-old, respectively. The analysis has been carried out through PCR amplification and subsequent *Hpa*I restriction analysis.

The results obtained indicated that in the patients' group neither the frequency of the A allele of the PROZ IVS6+79G/A polymorphism ($q=0.19$) nor the genotype distribution (GG=0.667, GA=0.291 and AA=0.042) were significantly different ($\chi^2 p>0.05$) from those obtained in controls (frequency of the A allele=0.20; GG=0.635, GA=0.325 and AA=0.040). All the groups analysed were in Hardy Weinberg equilibrium. There was also no statistical significance between both ischemic ($q=0.20$) and hemorrhagic ($q=0.15$) stroke patients and healthy controls.

From these results we conclude that the A allele of the PROZ IVS6+79G/A polymorphism is not a protective factor for stroke in our population.

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P1126. *SDHD* Variants Do Not Constitute a Risk Factor for Developing C-Cell Hyperplasia, or Sporadic Medullary Thyroid Carcinoma

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Medullary thyroid carcinoma (MTC) is a tumor that arises from parafollicular cells of the thyroid gland. MTC can occur sporadically (75%), or as part of inherited cancer syndromes (25%). In most cases hereditary MTC evolves from preneoplastic C-cell hyperplasia (CCH), so early detection of this pathology would evidently be critical. A recent study described a family with CCH as being attributable to the variant c.149A>G (H50R) in *SDHD*. This gene codes for one of the mitochondrial succinate dehydrogenase subunits and has been found altered, both in paraganglioma (PGL) and pheochromocytoma (PCC) families. We firstly studied *SDHD* in two families with hereditary non-RET CCH, and found no alterations related to the inheritance of this disease. We then investigated whether the H50R variant could be a risk factor in the sporadic development of MTC both in Spanish and English patients. We found no evidence that the presence of the H50R is strongly associated with the risk of sporadic MTC, though we did observe an association with age at diagnosis of MTC in Spanish H50R carriers that we did not find in English patients. Finally, we looked for evidence of CCH or any other thyroid disease in a panel of germ-line *SDH* (B or D) mutation carriers, and found none. We conclude that *SDHD* variants do not constitute a risk factor for developing CCH, or sporadic MTC. We also point to the necessity of confirming the pathogenicity of controversial variants, by means of analyze individuals coming from distinct populations.

P1127. CTLA4 alanine-17 polymorphism and spanish patients with Graves disease

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Graves disease (GD) is a pathology of autoimmune nature that is produced as result of a complex interaction among genetic and environmental factors. In the last years innumerable efforts have been done to define the genes of genetic susceptibility of this disease, nevertheless in multiple occasions the results have been scanty and even contradictory. Numerous studies have demonstrated the important role of the polymorphism of the gene CTLA4 in the susceptibility of this disease. The gene CTLA4 is placed in 2q33 and codifies the T cell receptor that modulates negatively the immune response disabling to the T-cells. The aim of the present work is to determine the contribution of the A/G dimorphism at position +49 in exon 1 of the CTLA4 gene to the disease. Fifty patients with GD and 50 unrelated healthy subjects from general population were analyzed as controls. The GD was defined as the presence of hyperthyroidism analytical (T4L raised and undetectable THS) together with two or more of the following criteria. Diffuse goiter, high titles of thyroid antibodies and the presence of ophthalmopathy. Restriction enzyme digestion with *BbvI* of polymerase chain reaction (PCR) amplified genomic DNA for the A/G dimorphism was used to analyze the polymorphism. Preliminary results suggest differences in the allele frequencies between the two groups GD and controls. For the G allele we found 48% in GD patients and 35% in controls. Our preliminary results show evidence of association of any of the CTLA4 gene polymorphism with the disease in Spanish patients.

P1128. The leucine to proline polymorphism at aminoacid 7 (Leu7Pro) in prepro-neuropeptide Y is more frequent in patients with major depression in a Danish population.

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There is increasing evidence from both animal and human studies that neuropeptide Y (NPY) plays a role in depression and anxiety. A polymorphism in the signal peptide of NPY that leads to substitution of leucine with proline (Leu7Pro) has previously been associated with hypercholesterolemia and alcohol dependence. The present study examined the distribution of the Leu7Pro polymorphism in a Danish population of patients with major depression (n=220) or panic disorder (n=121) and in a group of ethnically matched controls (n=730). We also studied the polymorphism in a group of schizophrenic patients (n=275). The Leu7Pro polymorphism was found to occur at a significantly higher genotype frequency in patients with major depression (10.5%; p<0.05, chi squared test) as compared to the control group (6.4%). Frequency was also higher in panic disorder (8.3%) and schizophrenic patients (8.7%), but this did not reach significance. In conclusion, the present study shows an association between the Leu7Pro polymorphism and depression, suggesting that the polymorphism could be involved in the patophysiology of depression in the Danish population.

P1129. Study for possible association of a polymorphism of the interleukin-6 gene promoter and premature coronary artery disease in the Greek population

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The interleucine-6 (IL-6) protein is a pleiotropic cytokine which play a central role in inflammation. The former was suggested to be involved in the pathogenesis of different diseases, such as atherosclerosis and coronary artery disease (CAD). In the present study we investigated the possible association between the -174G/C polymorphism of the promoter of interleukin-6 (IL-6) on risk of premature CAD in the Greek population. In a prospective case-control study, 129 CAD patients, documented by coronary angiography, aged under 58 years and 120 healthy controls were studied. To genotype the subjects we used the PCR-RFLPS method. The frequencies of GG, GC, CC genotypes were 0.66, 0.31, 0.03, respectively, in the patient group and 0.62, 0.31, 0.07, respectively, in the control group. The data between the two groups were analyzed by chi-square test. Our results showed that there was no patient/control significant differences and they suggest that there is no association of the IL-6 -174G/C promoter polymorphism with the risk of premature coronary artery disease in this Greek population.

P1130. Genetic analysis of the hypothesis of altered oligodendrocyte function in schizophrenia

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Background: Oligodendrocyte abnormalities have been implicated in schizophrenia by a diverse range of experimental approaches including gene expression analysis, neuropathology, and neuroimaging. With the aim of establishing whether such abnormalities are of primary aetiological relevance to schizophrenia pathogenesis, we are currently examining genes relevant to myelination and oligodendrocyte function for association with schizophrenia. **Methods:** Candidate genes are being examined in large case control and family based association samples using a combination of direct association analysis based upon *de novo* mutation detection and also by indirect association analysis based upon dense maps of database markers. **Results:** Nominally significant evidence (p<0.05) for association has been found for the *OLIG2* gene coding for a transcriptional factor that plays a role in terminal differentiation of oligodendrocytes. **Discussion:** So far, the evidence for association requires further analysis of more markers within the region as well as a replication in independent laboratories. Nevertheless, our data provide support for the hypothesis that oligodendrocyte function is relevant to schizophrenia pathogenesis.

P1131. Linkage analysis in a family with autosomal dominant nocturnal frontal lobe epilepsy

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a distinctive epilepsy syndrome characterized by short, partial seizures during non-REM sleep. Episodes often occur in clusters and localize to the frontal lobe. ADNFLE is inherited as an autosomal dominant trait with high penetrance. Mutations in the genes CHRNA4 (ENFL1 locus) and CHRNB2 (ENFL3 locus), encoding distinct neuronal nicotinic acetylcholine receptor (nAChR) subunits, have been associated with ADNFLE and account for only a minority of cases. A third genetic locus (ENFL2) has been identified but mutations linked to this form of the disease have not yet been found. Here we report on the results obtained by linkage analysis of the known loci performed on a family with ADNFLE coming from Calabria (Southern Italy). The affected members, previously analyzed by sequencing all coding regions and exon-intron boundaries, showed no mutations in CHRNA4 and CHRNB2 genes. The linkage analysis, performed on fifteen members of the family, confirmed that these genes were not involved in the pathogenesis of the disease. Moreover, we tested the involvement of the ENFL2 locus containing the CHRNA5/A3/B4 gene cluster and the data obtained allowed us to exclude any association between this locus and ADNFLE in our family. Currently, further linkage analyses are underway to determine whether genomic regions coding for additional nAChR subunits are associated to ADNFLE in the examined family.

P1132. Associated transmission chromosomes 3+11 in patients with autosomal recessive benign erythrocytosis from Chuvashia.

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Several years ago we have demonstrated linkage of the autosomal recessive benign erythrocytosis that has a high incidence in Chuvashia on 11q23 (Lod = 6.61) but gene of this disease was not found. Using genome-wide searching Ang et al, (2002) determined C598T change in *VHL* gene (3p26-25) which result in Arg200Trp mutation in homozygosity in all individuals affected with Chuvash polycythemia. *VHL* functions as a recessive tumor suppressor gene and most of mutations in this gene were found in von Hippel-Lindau syndrome in heterozygosity in all tissues but a second mutation found in tumor only. None of erythrocytosis patients who have homozygous Arg200Trp mutation in *VHL* gene and none of their heterozygous relatives have not any symptoms of von Hippel-Lindau syndrome. On the other hand we revisited the transmission disequilibrium between chromosomes 3 and 11 in Chuvash polycythemia patients. We have investigated patients group, their healthy sibs and independent control group for associated transmission chromosomes 3 and 11 from parents to offspring by haplotype analysis at D3S1597 - D3S1263, and D11S4111 - D11S4127 - D11S1356 markers. We determined that in 75% cases affected patients get from their parents the same chromosomes 3 and 11. This value was only 44% ($\chi^2=16.14$; $p<0.001$) for sibs and 43% ($\chi^2=17.91$; $p<0.001$) for control group. We concluded that 3p25 and 11q23 loci transmit not independently from each other in patients group. This fact may be connecting with modification locus on chromosome 11, which is influential in *VHL* gene mutation manifestation at Chuvash benign erythrocytosis.

P1133. Association of polymorphism within the tumour necrosis factor gene and childhood asthma in the north-west region of Russia.

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BACKGROUND: Tumour necrosis factor alpha (TNFalpha) is a potent modulator of immune and inflammatory responses, and has been implicated in a variety of autoimmune diseases, including asthma. Interindividual variation in TNFalpha levels could be genetically determined and polymorphism within the TNF gene have been

associated with differences in TNFalpha production.

OBJECTIVE: To investigate the association of differences in asthma-related phenotypes with two biallelic polymorphisms: G to A substitution at position -308 and G to A substitution at position -238 of the TNFalpha gene promoter.

METHODS: The regions of interest were amplified from genomic DNA by means of specific primers and PCR. The restriction enzyme digestion was used for genotyping individuals for the TNFalpha -308 and -238 polymorphisms. A case-control analysis was performed for 83 asthmatic and 117 non-asthmatic unrelated children.

RESULTS: TNFalpha -238A allele was present at a significantly low frequency in the patients group (OR=0.21; $P=0.03$). In contrast, an increased frequency of -308 A allele was found in the asthmatic group as compared to the control group (OR=2.5; $P=0.03$). It should be noted, that the frequency of -308 A allele in the females from study group was three times more, than in the female from control group (15% and 4% respectively).

CONCLUSION: -308A TNF promoter polymorphism might be suspected as genetic predisposition factor to asthma especially for females in childhood age. In contrast, -238A allele TNF promoter polymorphism has same protective effect to asthma.

P1134. Linkage analysis of congenital motor nystagmus in Russian family

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X-linked motor nystagmus is a common oculomotor disorder characterized by repetitive uncontrollable ocular oscillations with onset typically at birth. X-linked, autosomal-dominant and autosomal-recessive inheritances have been described. The molecular defect of the disorder is still unknown.

Three loci for X-linked nystagmus have been mapped to Xp11.4-p11.3 and Xq26-q27. CDR1, SOX3, SLC25A14, SLC9A6 and FGF13 (Xq26-q27) were studied as candidate genes, but no mutation was detected. To map and precise the genetic interval in which the gene of X-linked nystagmus is placed we investigated 45 persons from five-generation Russian family with X-linked inherited motor nystagmus, in which 9 individuals were affected (6 males and 3 females); 14 individuals, including 7 affected (4 males and 3 females) were involved in this DNA-study. The penetrance among female carriers is incomplete. Genotyping and haplotype analysis were performed using 20 polymorphic microsatellite markers from X chromosome: NDPCA (Xp11.4-p11.3) and DXS1062, DXS1192, DXS8093, DXS8044, DXS1047, DXS8071, DXS1206, DXS8068, DXS994, DXS8074, DXS8050, DXS8038, DXS8094, DXS6789, DXS6805, DXS1001, DXS1227, DXS1193, DXS8087 (Xq26-q27). The haplotype analysis revealed no evidence of linkage with marker NDPCA from the short arm of X chromosome in this family. Evaluation of haplotypes from the long arm of X chromosome showed that all affected individuals had the same haplotype with markers DXS1047, DXS8068, DXS994, DXS8071 and DXS8074. Multipoint linkage analysis using these four polymorphic markers estimated the maximum multipoint LOD-score of 2.6. Other markers from this region are in study for the fine mapping. This work was supported in part by President's RF grant MD-2456.2004.4

P1135. Genotype / phenotype correlation in "atypical" CF clinical pictures

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The clinical manifestations in Cystic Fibrosis are quite heterogeneous and descriptions of patients with atypical features have recently appeared. These patients are commonly Pancreatic Sufficiency, they show normal or borderline (40-60mMol/l) chloride levels in the sweat test and they have symptoms involving a single organ. One of the two CF mutations is in fact surely not severe, resulting in a residual CFTR

protein expression and function. The wide range of symptoms makes CF diagnosis more difficult and delayed. In this study we analysed the genotype and phenotype in 117 cases with monosymptomatic manifestations. The CFTR coding tract screening was performed using DHPLC technique and direct sequencing.

The clinical presentation includes: 46 patients with respiratory involvement, 26 CBAVD, 8 with idiopathic pancreatitis, and 37 cases with clinical features not yet valuable (neonates with positive IRT and with borderline sweat test).

The molecular results led us to identify:

53/117 (45.3%) compound heterozygous

57/117 (48.7%) heterozygous; 19 of them with IVS8- T5/TG12 haplotype

2/117 (1.7%) homozygous for mild mutations

5/117 (4.3%) none CFTR mutation; one of them with homozygous IVS8- T5/TG12 haplotype.

The haplotype IVS8- T5/TG12 seems to be related to the pathogenesis of CF when occurs in these genetic pattern.

These data and recent publications seem to prove that the high degree of the clinical heterogeneity in this pathology is related to the large spectrum of genetic variability which cause, rather than a loss of protein function, a "gradient" of CFTR dysfunction.

P1136. The glutation-S-transferaseT1 and M1 genes polymorphism and risk for early recurrent miscarriage.

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Objective: The pathogenesis of recurrent miscarriage is complex, presumably involving the interaction of several genetic and environmental factors. The frequency of clinically recognized spontaneous abortion in the general population has been estimated to range between 15-20%. The aetiology of early recurrent miscarriage remains unclear, but it may be related to a possible genetic predisposition together with involvement of environmental factors. Polymorphisms in genes can lead to differences in the level of susceptibility of individuals to potentially adverse effects of environmental influences, such as chemical exposure, on prenatal development or male or female reproductive function. The glutathione-S-transferase genes responsible for xenobiotic conjugating enzymes of Phase II detoxification system and have been implicated as risk factors for recurrent embryo loss in early pregnancy.

Materials and methods: Genetic polymorphisms of two genes GSTM1, GSTT1 in 205 patients with a history of two or more unexplained first-trimester recurrent miscarriages and 76 women who had one or more normal pregnancies and no obstetric complications or history of miscarriage were studied by PCR.

Results: 35 of the 205 women with recurrent miscarriage (17,1%) and 5 of the 76 control women (6,6%) were homozygous for the glutathione-S-transferase T1 null and glutathione-S-transferase M1 null alleles (GSTT10/0/GSTM10/0). The relative risks of first-trimester recurrent miscarriages in carriers of the GSTT10/0/GSTM10/0 was 2,92 (95% CI=1,14-7,48). 28,7% of the women and 14,5% of the control have genotypes GSTT10/0/ GSTM1+ (relative risk, 2,38 (95% CI=1,14-4,96).

Conclusions: Both the genotypes GSTT10/0/GSTM10/0 and GSTT10/0/ GSTM1+ are associated with first-trimester recurrent miscarriage.

P1137. Polymorphisms of genes predisposing to cardiovascular disorders in group of patients with the steroid-resistant nephrotic syndrome

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The steroid-resistant nephrotic syndrome (SRNS) is autosomal recessive disease with an early childhood onset of proteinuria, rapid progression to the end-stage renal disease and focal segmental glomerulosclerosis. Mutations in NPHS2 gene, mapped to 1q25-31, are the cause of the SRNS.

We have examined 30 children with biopsy proven SRNS in age ranged from 9 to 17 years (SRNS group) and 50 healthy children of the

same age (control group). We have used PDRF and PDAF methods for definition of the allele frequencies of several genes: PON1(R192Q), AGT(M174T and M235T), ACE(ID 287 bp, intron 16), AGTR1(A1166C), FVII(R353Q and -353 I/D 10bp), PAI(-675 4G/5G), MTHFR(C677T), NOS3(T786C, 27 bp repeat, intron 4- 4a, 4b and E298D).

The distribution of allele frequencies for each group was corresponding to Hurdy-Weinberg equilibrium. The distinction of allele frequencies between SRNS and control groups was valued using χ^2 criterion.

We have determined higher frequencies of alleles: M174 and M235 (AGT); I (ACE); A1166 (AGTR1) and the lower frequencies of A2 (323 ins 10bp) and Q353 in FVII gene in SRNS group. The differences of frequencies are significant.

We have also valued the association between genotypes and the disease using odds ratio criterion. The positive significant association was found between the SRNS and several genotypes: T174|M174 and T235|M235 for AGT; I|I for AGTR1; A1|A1 and R|R for FVII. The reliable difference between SRNS and control groups have not been found for another allele variants of genes associated with cardiovascular disorders

P1138. Refined mapping of autosomal dominant, adult-onset, leukodystrophy (ADLD) in a Swedish kindred

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The clinical physiopathology of autosomal dominant, adult-onset, leukodystrophy (ADLD) is characterised by a slowly progressive neurodegenerative disorder with a symmetrical, widespread myelin loss in the white matter. The initial symptoms are recognized by bowel/bladder dysfunction, loss of fine motor skills, ataxia, and the disease are lethal within ~20 years from onset. We have mapped the ADLD gene to a small interval (3 Mbp, 1.6 cM) on chromosome 5q23-q31 in a Swedish kindred, extending over five generations. This refined mapping restrict the ADLD mutation to a region spanning 14 genes. Further mapping is in progress using additional microsatellites, SNPs and DNA sequencing of affected and unaffected individuals.

P1139. Gene polymorphisms of neuronal and endothelial nitric oxide synthases and colonization of *Pseudomonas Aeruginosa* in patients with Cystic Fibrosis

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Objective: NO is endogenously produced by a group of enzymes, the NO-synthases (NOSs). There are three isoforms of NOS encoded by different gene: NOS1, iNOS, eNOS. These genes are constitutively expressed in cells of the respiratory tract. Patients with cystic fibrosis (CF) have decreased concentrations of expired nitric oxide (FeNO) as compared to healthy individuals. A number of factors, including viscous mucus as a diffusion barrier for airway NO, consumption of NO by bacterial enzymes, and decreased NO production have been hypothesized to account for these low levels of FeNO.

Materials and methods: Genetic polymorphisms of the NOS1 gene and of the gene eNOS were studied by PCR-based assays in 37 CF patients and 69 healthy individuals.

Results: We examined the relationship between the size of the AAT repeat polymorphism of the NOS1 gene, the 4a/4b polymorphism of the eNOS gene and colonization of the airways with *Ps.aeruginosa*. Colonization of the airways with *Ps.aeruginosa* was significantly ($p<0.05$) more common in CF patients with high numbers of AAT repeats in the NOS1 gene (≥ 12) and 4b/4b eNOS.

Conclusions: The results provide the evidence that the polymorphic variants of the NOS1 gene and the eNOS gene are associated with *Ps.aeruginosa* colonization of airways in CF patients. We could suggest that production of NO is one of mechanisms of local lungs protection. Because of this the decrease of NO production (FeNO) leads to decrease of antibacterial lungs protection and more favourable conditions for the colonization of *Ps.aeruginosa* could be formed.

P1140. A Novel Locus for Recessive Syndactyly Maps to Chromosome 17p13.3.

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Non-syndromic syndactyly is a common congenital malformation showing webbing of fingers and/or toes. The malformation can be unilateral or bilateral, and the fusion within the web may be cutaneous or bony.

We revise the systematics of syndactylies. The majority of them show an autosomal dominant mode of inheritance except Cenani-Lenz syndactyly which follows the recessive model.

Previously we reported a novel syndactyly in a consanguineous Pakistani family segregating as an autosomal recessive entity with a unique combination of clinical features: mesoaxial reduction of fingers with synostosis of 3rd and 4th metacarpal bearing single phalanges, clinodactyly of 5th fingers and preaxial webbing of toes. Three similarly affected patients with this distinguished phenotype were observed in a large inbred Turkish family. We now localize the phenotype in the Pakistani and Turkish family to chromosome 17p13.3 (multipoint lod score 5.23).

The identification of a single locus for a similar complex hand-foot malformation in two families with distinct ethnical backgrounds gives evidence for a new form of syndactyly. We propose to name this phenotype mesoaxial synostotic syndactyly with phalangeal reduction (SDMS, type IX syndactyly, Malik-Percin type).

The mapping of this novel locus contributes to the clinical and genetic delineation of syndactylies. Recruitment of more families with this phenotype might help to narrow down the candidate region, and the cloning of the gene would provide insight into the complex process of limb development.

P1141. Genomewide scan for affective disorder susceptibility loci in families of a northern Swedish isolated population

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We analyzed 9 multigenerational families with affective spectrum disorders ascertained in the geographically isolated population of Västerbotten in northern Sweden. This northern Swedish population, that originated from a limited number of early settlers approximately 8000 years ago, is genetically more homogeneous than outbred populations. Therefore, its use maximizes the probability of detecting genes by genetic approaches.

In a genomewide linkage analysis we identified three chromosomal loci with multipoint LOD scores (MPLOD) ≥ 2 at 9q31.1-q34.1 (MPLOD 3.24), 6q22.2-q24.2 (MPLOD 2.48) and 2q33-q36 (MPLOD 2.26) under a recessive affected-only model. Follow-up genotyping applying a 2 cM density STR map confirmed linkage at 9q31.1-q34.1 (MPLOD 3.22), 6q23-q24 (MPLOD 3.25) and 2q33-q36 (MPLOD 2.2) and resulted in candidate regions of 10 cM, 3 cM and 23 cM respectively.

In an initial analysis aiming at identifying the underlying susceptibility genes, we focused our attention on the 9q locus. We finemapped this region at a 200 kb STR density resulting in a MPLOD of 3.70. The candidate region further decreased to 4 cM. Genealogical studies showed that 3 families linked to chromosome 9q descended from common founder couples 10 generations ago. These families enabled the identification of an identical by descent (IBD) haplotype in patients reducing the 9q candidate region to 1.6 Mb. Further, the shared haplotype was observed in 4.2% of BPI patients but not in control individuals in a patient-control sample from the Västerbotten isolate.

These results suggest a susceptibility locus on 9q31-q33 for affective disorder in this common ancestral region.

P1142. SNP haplotype analysis of TPH2 in northern Swedish unipolar and bipolar patient/control populations.

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Recently, Walther and colleagues (2003) identified the gene for a novel tryptophan hydroxylase (TPH) isoform, TPH2 that is encoded on chromosome 12 and is exclusively expressed in the brain. TPH is the rate-limiting enzyme for the production of serotonin from tryptophan. Serotonin is a key neurotransmitter in the central nervous system, and dysfunction of the serotonergic system has been implicated in several psychiatric diseases. We have investigated the potential influence of polymorphisms at the TPH2 locus on the development of unipolar affective and bipolar affective disorder. From the HapMap database we selected 6 htSNPs representing haplotypes with a minor allele frequency above 5%. These htSNPs plus one putative promoter SNP, (Kennedy et al. *Neuropsychiatric Genet.*, 2003), were genotyped in a sample of 136 patients with DSM-diagnosed unipolar affective disorder, 182 patients with DSM-diagnosed bipolar affective disorder and 364 healthy individuals, all of northern Swedish descent.

Out of 7 genotyped SNPs, we identified one SNP that was significantly associated with unipolar affective disorder and one SNP with bipolar affective disorder. One SNP, located in the putative promoter region, showed a p-value of 0.027 (promSNP) in our unipolar affective sample and one htSNP (rs10748185) showed a p-value of 0.048 in our bipolar affective sample. Haplotype analysis provided supportive evidence for an involvement of genetic variation at the TPH2 locus with unipolar affective disorder (p-value=0.031) and bipolar affective disorder (p-value=0.004). For both, unipolar and bipolar affective disorder, the significant haplotypes that we've found were consistent with protective factors.

P1143. Abnormalities of ceruloplasmin gene expression in mammary gland associated with structure of promoter region

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Copper is essential for growth and development of newborns and simultaneously it is a high toxic. In milk, copper is packed into tissue specific ceruloplasmin (Cp). Its concentration sharply drops during first month of lactation. Likewise that copper level in newborn diet intake is controlled by Cp gene activity in mammary gland. Milk Cp and copper concentration in 5 days after postpartum and Cp promoter region structure (genetic marker) were analyzed to detect Cp sequences that effect in the CP gene expression regulation in mammary gland cells. The samples of 41 DNAs were analyzed by PCR-method using 17 pairs of overlapping primers to 5'-region 4000 bp from +192 bp. Simultaneously, skimmed milk Cp and copper concentration at the 1st and 5th days after postpartum were measured by rocket immunoelectrophoresis and atomic absorptive spectrometry respectively. Two cases of abnormalities of Cp gene expression were revealed. In a one case milk Cp concentration did not decrease during 5 days and even doesn't change after 1.5 year lactation. At the same time blood Cp concentration corresponded to average value. A substitution ¹⁹⁹⁶A/C in site for transcription factor C/EBPbeta was found by automatic sequencing. In another case very high Cp concentration in skimmed milk was found (40 versus 14 mg/100 ml). Sequencing indicated 90(bp)-insertion in (-1639)(-2294) bp region, which included coterminous of estrogen receptor site and site for binding YY1 protein.

P1144. Polymorphisms of the endothelial nitric oxide synthase gene in placentas with intrauterine growth restriction (IUGR).

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Objective: Intrauterine growth restriction is a syndrome involving dysfunction of vascular endothelium and imbalance between

endothelium derived constricting and relaxing factors. Recent evidence suggest a major role endothelium-derived nitric oxide (NO) plays in the regulation of vascular resistance during normal pregnancy and pregnancy with IUGR. NO is a potent vasodilator generated by the catalytic action of endothelial nitric oxide synthase (eNOS) in placenta. Mutations in the endothelial nitric oxide synthase (eNOS) gene may be associated with abnormal nitric oxide (NO) production. The 4a/4b genotype is known to be associated with reduced levels of NO metabolites (25% decrease).

Materials and methods: We collected and performed PCR the 4a/4b polymorphism of the eNOS gene in placentas of 33 patients with IUGR and 35 women with one or more normal pregnancies and no obstetric complications.

Results: The frequencies of alleles among samples with IUGR and controls were 86,3% and 82,8% respectively, for allele 4b (wild type), and 13,7% and 17,2%, respectively, for allele 4a (mutant). No association between allele 4a and IUGR was found ($P>0.05$, OR 1.31). The distribution of genotype were not significantly different between the studied groups (4b/4b: 72,7%; 4b/4a: 27,3%; 4a/4a: 0%) and the control group (4b/4b: 68,6%; 4b/4a: 28,6%; 4a/4a: 2,8%) ($P>0.05$). Conclusions: The 4a/4b polymorphism of the eNOS gene is not implicated in increased risk of intrauterine growth restriction.

P1145. The study of the β_2 -adrenergic receptor polymorphisms in asthma patients and healthy donors from the Volga-Ural region of Russia

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Bronchial asthma (BA) is a complex disorder, the development of which is determined by the interaction between genetic and environmental factors. To date, numerous DNA-loci and candidate genes have been reported to show linkage and association to asthma. The human β_2 -adrenergic receptor (ADRB2) is a target of β_2 -agonist drugs used for bronchodilatation in asthma and ADRB2 gene may be examined as potential asthma-susceptibility gene.

This study reports the results of the investigation of the β_2 -adrenergic receptor Arg16Gly and Gln27Glu polymorphisms in asthma patients and healthy donors from Volga-Ural region of Russia. The asthma group consisted of 140 patients; the control group included 203 unrelated nonasthmatic subjects. We have determined that the frequencies of Gly16 allele and Gly16/Gly16 genotype were noticeably higher in patients with atopic asthma (67% and 48%, respectively) in comparison with 57% and 27% for infection-dependent asthma. The frequency of Glu27 allele was significantly greater in patients with atopic asthma (51,7%) compared with infection-dependent asthma (40%). The proportion of Glu27 homozygotes was 27,6% in atopic asthma group and 16,2% in infection-dependent asthma group. Strong allelic association was observed between Gly16 allele and Glu27 allele and between Arg16 allele and Gln27 allele ($p<0.05$). The combination of Gly16/Gly16 and Glu27/Glu27 genotypes occurred more frequently in atopic asthma patients (27,6%) than in control group (15,2%). OR=1,8 (95%CI 0,79-3,64).

The data of this study revealed differences in the distribution of polymorphic alleles and genotypes of the ADRB2 gene between asthma patients and healthy donors and between different clinical forms of bronchial asthma.

P1146. Association of ACE I/D polymorphism with hypertension in Croatia and an isolate

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The angiotensin-converting enzyme (ACE) takes part in the renin-angiotensin system that is one of the main regulatory systems in blood pressure homeostasis. Its influence on hypertension has been widely explored in various populations, but the results are still conflicting. The insertion/deletion polymorphism (ACE I/D) in intron 16 of the ACE gene is easily detectable and seems to be a good marker for ACE

influence on various phenotypes. We studied ACE I/D polymorphism and hypertension in the general Croatian population, where we found the homozygous deletion genotype is associated with hypertension. This influence seems to be specially pronounced in men younger than 40 years. These results are compared with the results of testing the ACE I/D association with hypertension in one of the Croatian isolates - the island of Vis, which is characterized by a high prevalence of hypertension as well as high levels of inbreeding.

P1147. Genetic analysis of Bartter and Gitelman Syndrome in the Italian population

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Bartter's syndrome includes a group of autosomal recessive disorders caused by loss of function mutations in one of four genes: SLC12A1, KCNQ1, CLCNKB and BSND encoding respectively: the sodium potassium-chloride cotransporter NKCC2 (Bartter type I), the inwardly rectifying potassium channel ROMK (Bartter type II), the basolateral chloride channel CIC-Kb (Bartter type III) and the Barttin protein, an essential subunit of CIC-Kb. The latter phenotype is associated with sensorineural deafness (Bartter type IV). All of them are expressed in the thick ascending limb of the Henle loop. Gitelman's syndrome is a variant of Bartter's syndrome, and is caused by mutations in the gene SLC12A3, encoding the sodium-chloride cotransporter NCCT. This protein is expressed in the distal convoluted tubule of the nephron. The aim of our study is the characterisation of the molecular defects of patients without sensorineural deafness, clinically defined as Gitelman/Bartter syndrome. Our patient population includes a cohort of 91 subjects. On the basis of the mutations identified in the SLC12A1, KCNQ1, CLCNKB and SLC12A3 genes, 55 patients showed mutations on both alleles (22 homozygous and 33 compound heterozygous mutations). On the basis of detected mutations we could define that 6 patients were affected with Bartter type I syndrome, 4 with Bartter type II, 13 with Bartter type III, and 32 with Gitelman syndrome. The complete clinical phenotype and the genotype-phenotype correlation will be discussed.

P1148. Polymorphism of the glutation-S-transferase P1 gene and early recurrent miscarriage.

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Objective: The clinical management of repeated early pregnancy wastage focuses on several etiologic factors (i.e., genetic, medical, immunologic, endocrine, psychogenic, environmental, occupational, infectious, and uterine), which have been noted to result in repeated pregnancy wastage. Genetic variability in biotransformation enzymes could be associated responsible for predisposition differences to recurrent miscarriage. The glutatione-related detoxification system plays an important role to ensure an uncomplicated pregnancy outcome. The components of the glutatione-related detoxification system are equally distributed among the different cotyledons in the human placenta.

Methods: Polymorphisms in the genes of glutation-S-transferases P1 were assessed in 94 nonpregnant women with a history of early (≤ 16 weeks gestation) recurrent miscarriage (two or more spontaneous abortions) and 76 healthy control women.

Results: The frequencies of alleles in women with recurrent miscarriage and controls were 73.4% and 70.4%, respectively, for the A allele (wild type), 18.1% and 22.4%, respectively, for the B allele (mutant), and 8.5% and 7.2%, respectively, for the C allele (mutant). The distribution of the genotypes for the glutation-S-transferase P1 gene was not significantly different between the study group and the controls.

Conclusions: We conclude that the presence of mutant alleles of glutation-S-transferase P1 gene is not a risk predictor in women with a history of early recurrent miscarriage.

P1149. Immunogenetic studies in Hungarian patients with idiopathic inflammatory myositis

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Objectives Idiopathic inflammatory myopathies (IIM) are rare systemic autoimmune diseases, characterized by progressive weakness of the skeletal muscles. As in other autoimmune diseases, the immune process seems to be induced by environmental factors in genetically susceptible individuals. Our aim was to extend the knowledge of ethnogeographic studies about HLA association of myositis. We investigated the presence of the HLA-DRB1, -DQA1 and DQB1 alleles in Hungarian patients with different forms of myositis.

Patients and Methods 93 patients with myositis and 54 healthy unrelated controls were enrolled in the study. HLA genotyping was performed by polymerase chain reaction with sequence specific primers technique to determine different allelic groups (Olerup-SSP). We also assessed the relation with phenotypic features, e. g. clinical manifestations, autoantibodies and clinical course.

Results Among all patients with any forms of myositis, frequency of HLA-DRB1*0301 and the linked -DQA1*0501 were significantly increased compared to controls. HLA-DQB1*05 alleles were also found to be increased. A significantly increased frequency of DRB1*01 and DRB1*07 were observed in patients with myositis in overlap with another systemic autoimmune disease. The HLA-DRB1*12 appeared to provide protection against myositis. We did not find alleles that predispose for systemic manifestations. Patients with the myositis specific Jo-1 autoantibody carried DRB1*03 and DQB1*04 in higher frequency. According to the clinical course significant differences in the genotype could not be detected.

Conclusion Concerning the most important genes that provide susceptibility to myositis, we found similar associations of HLA Class II alleles in Hungarian patients to other Caucasian populations.

P1150. A genome-wide search of Late-Onset Alzheimer's disease using homozygosity mapping shows evidence of a new locus on chromosome 3.

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Alzheimer's disease (AD) often segregates as a dominant trait in families but a recent study, performed in an inbred community, suggested a recessive mutation (Farrer LA, et al, 2003). We studied 10 individuals affected with probable late-onset AD from 5 inbred families coming from a recently genetically isolated Dutch population. These individuals could be connected to a common ancestor in 8 generations, suggesting the presence of a recessive mutation involved in the onset of the disease. In order to identify genes implicated in the pathophysiology of AD, we performed a genome screen of AD using 420 markers on these 10 individuals and mutations on APP, PSEN1 and PSEN2 genes were excluded by direct sequencing. All probands carried the APOE ε3/4. Homozygosity mapping of the 10 individuals yielded two peaks on chromosome 3 with multipoint LOD scores of 3.1 (from marker D3S3681 to D3S1569) and 3.4 (from marker D3S3626 to D3S1262) respectively. After fine mapping these regions with 10 extra markers, the first peak could be narrowed down from ~50 to 19 cM with a maximum LOD score of 3.9 (from marker D3S1292 to D3S1549) and the second peak could be narrowed down from ~30 to 5.8 cM with a maximum LOD score of 3.2. This region included the gene Butyrylcholinesterase (BCHE). Direct sequencing of the coding region of this gene showed no variants that could explain the presence of the disease. These findings suggest the presence of a new locus on chromosome 3, which may involve a recessive mutation.

P1151. Renin Angiotensin System polymorphisms and breast cancer risk

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Angiotensin II has growth factor properties and may be involved in the etiology of breast cancer. The Insertion/Deletion (I/D) polymorphism in the Angiotensin-Converting-Enzyme (ACE) gene determines levels of ACE and the M235T polymorphism in the Angiotensinogen (AGT) gene accounts for the variability of AGT plasma concentrations and may produce an increase in Angiotensin II levels. These two polymorphisms were genotyped in 4117 women participating in the Rotterdam Study, including 144 patients with breast cancer. At baseline, information concerning risk factors was obtained by an interview. First, we performed a logistic regression analysis to assess the risk of breast cancer yielding an odds ratio (OR) of 1.86 (95% Confidence Interval [CI] = 1.06-3.27, p-value = 0.03) for ACE DD carriers and 2.01 (95% CI: 1.34-3.01, p = 0.001) for TT carriers of the AGT M235T polymorphism. Second, a Cox survival model was fitted and showed that breast cancer free survival by ACE genotype was significantly reduced in DD compared to II carriers (HR = 1.80; 95% CI: 1.07-3.01, p-value = 0.03). We observed a similar effect for TT carriers (HR = 1.80 95% CI: 1.21-2.65, p-value = 0.002). These findings were independent of known risk factors. Our results suggest that the ACE and AGT proteins and perhaps the Renin Angiotensin System in general, play a role in breast cancer risk in postmenopausal women.

P1152. The locus for ichthyosis prematurity syndrome is restricted to 231 kb on chromosome 9q34.11

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Autosomal recessive congenital ichthyosis (ARCI) is a group of keratinisation disorders, where ichthyosis prematurity syndrome (IPS) is included. IPS is rare and almost exclusively present in a restricted region in the middle of Norway and Sweden, which indicates a founder effect for the disorder. We recently reported linkage of IPS to chromosome 9q34 and we present here the subsequent fine-mapping of this region with known and novel microsatellite markers. A haplotype associated with IPS was identified within the linked region on chromosome 9q34. This haplotype spans approximately 3.4 Mb and support previously suggestions of an ancient founder mutation. Based on the average length of the haplotype in IPS patients, we calculated the age of a founder mutation to at least 1500 years. The haplotype contain a core region of 231 kb consisting of three marker alleles shared by 95% of the affected individuals. This core haplotype restricts a region with seven known genes of which six are expressed in mature epidermal cells.

P1153. Association of some vascular genetic markers with different forms of preeclampsia

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Preeclampsia is a multifactorial disease with unknown genetic compound. One of the most important clinical parameters of preeclampsia is increased blood pressure. There are some major genes, which products regulate vascular tone. In this study an analysis of the association between polymorphism of some vascular genes and the development of preeclampsia was carried out.

I/D polymorphism of the angiotensin-converting enzyme (ACE), 4a/4b polymorphism of the endothelial NO-synthase (eNOS), I/D polymorphism of the tissue plasminogen activator (PLAT) and 4G/5G polymorphism of the plasminogen activator inhibitor type 1 (PAI-1) genes were studied by PCR-RFLP assay.

There was no significant difference between preeclamptic patients (n=117) and control group (n=79) in frequencies of genotypes and alleles of the ACE, eNOS, PLAT and PAI-1 genes. However the

frequency of genotype I/I of the ACE gene significantly increased in preeclamptic women with background arterial hypertension (27.5%) as compared to controls (10.3%, $p<0.05$). The frequencies of 4G allele of the PAI-1 and 4b allele of the eNOS gene were significantly higher in patients with mild (75.8%) and severe (100%) uncomplicated preeclampsia, respectively, as compared to control group (58.2%, $p<0.05$ and 80.4%, $p<0.05$). Moreover the frequency of D allele of the PLAT gene was significantly higher in the group of patients with preeclampsia (67.5%) as compared to population (54.2%, $p<0.005$). The differences in polymorphic variants of particular genes could be an evidence of the presence of several different disorders, joint by generic term "preeclampsia".

P1154. Analysis of insertion/deletion polymorphisms of angiotensin converting enzyme and bradykinin receptor genes (ACE and BKR2) in children and adolescents with arterial hypertension from North-West region of Russia.

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Renin-angiotensin-bradykinin system (RABS) is a regulator of arterial (blood) pressure and water-salt balance in human. Genetic polymorphism of this system is associated with cardiovascular diseases. The main aim of our investigation was to study the distribution of frequencies of RABS genes in children and adolescents with arterial hypertension (individuals of 9-18 age, N=176) and in population group (7-17 years, N=117) of North-West of Russia. On the basis of blood pressure load we have subdivided group with arterial hypertension into three groups: 1) without confirmed hypertension (<25%), 2) with labile hypertension (25-50%) and with stable hypertension (>50%).

The polymorphisms of the ACE gene (I/D) and the BKR gene (I/D) were studied by PCR analysis. Distribution of genotype frequencies between groups were compared by F-test. The distribution of relevant frequencies of polymorphisms for the ACE and the BKR genes was similar in children and adolescents with hypertension compared to population group. The division of groups by sex was not found out any significant differences in distribution of genetic polymorphisms between patients with hypertension and population group.

Interestingly that patients with labile hypertension had significantly lower frequency of DD genotype of ACE gene compared to children and adolescents without confirmed hypertension (22% and 50%, respectively; $p=0.04$) and population group (22% and 38%, respectively; $p=0.03$) but there was no difference as compared to patients with stable hypertension (22% and 33%; $p=0.27$).

It might be speculated that I/D polymorphism of ACE gene could be associated with labile hypertension in children and adolescents.

P1155. Multipoint linkage analysis for a very dense set of markers

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Multipoint linkage methods are powerful tools that are often employed as the first means to discover alleles affecting liability to diseases. With the advent of dense marker maps, linkage disequilibrium (LD) between markers is inevitable and it comes at the cost of bias and increased rate of false-positive findings for linkage analyses that assume alleles of different markers are independent. I propose a "multipoint on subsets" method that avoids this issue by partitioning the markers into interlaced and non-overlapping subsets. Each subset is analyzed separately, their statistics are then averaged, and the resulting average is normalized by its estimated standard deviation. In addition to being robust to the challenges induced by dependent marker alleles, data simulated under linkage equilibrium show that the proposed method does not suffer any loss of power when compared to traditional methods.

P1156. Association of protein-tyrosine phosphatase N1 gene polymorphisms with type 2 diabetes and insulin resistance

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Protein-tyrosine phosphatase-1B (PTP-1B) protein is encoded by the PTPN1 gene on 20q13. PTP-1B negatively regulates insulin signaling by dephosphorylating the insulin receptor. We have carried out an association analysis of 35 noncoding SNPs spanning the 161 kb genomic region including the PTPN1 gene. SNPs were assessed for association with type 2 diabetes (T2DM) in two independent collections of Caucasians with T2DM and two matching control groups. Significant evidence for association is observed, especially SNPs spanning the 3'-end of intron 1 of PTPN1 through intron 8 (P-values 0.043-0.004 in one case-control set and 0.038-0.002 in a second case-control set). Analysis of combined case-control data increased the evidence of association with T2DM (P=0.005-0.0016). All associated SNPs lie in a single 100 kb haplotype block that encompasses the PTPN1 gene. Haplotype analyses identified a significant difference between cases and controls (P=0.0035-0.0056) with one common haplotype contributing strongly to the evidence for association with T2DM. Odds ratios are approximately 1.3 for risk haplotypes. PTPN1 was also evaluated for association with measures of glucose homeostasis in 811 Hispanic subjects in the IRAS Family Study. All SNPs with minor allele frequencies > 0.1 are significantly associated with insulin sensitivity index (SI; P-values=0.044-0.003) and fasting glucose (P-values=0.029-<0.001); there was no evidence of association with acute insulin response (a measure of β -cell function). In haplotype analysis, the diabetes risk haplotype is associated with lower SI and higher fasting glucose (P=0.005 and P=0.00003, respectively). These results suggest that PTPN1 is a significant contributor to T2DM susceptibility and insulin resistance.

P1157. SLC22A4 and SLC22A5 variants are associated with perianal Crohn's disease in Belgian patients.

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Functional variants of the SLC22A4 (1672C→T) and SLC22A5 (-207G→C) genes coding for cation transporters, were recently shown to be associated with Crohn disease (CD). A study of Armuzzi et al.(2003) presented evidence suggesting that the association might be specific for a CD subtype, namely the perianal form. In this study, we test the association of these 2 variants with clinical CD subtypes in a cohort of Belgian patients.

We studied a cohort comprising 619 CD patients including 209 trios. Untransmitted parental chromosomes from the trios were used as controls in addition of 94 ethnically matched controls. Genotyping was done by using the Taqman technology. Allelic frequencies in cases and controls were compared using a one-sided chi-squared test. Maximum likelihood haplotype frequencies were estimated using an EM algorithm. We found no evidence for association of C1672T and G-207C with CD neither when considering each marker alone ($p=0.235$; $p=0.113$ respectively), nor when considering them as a haplotype. However, when we classified patients according to perianal disease at the time of diagnosis ($n=72$), the corresponding analysis yielded strong evidence for an association with both markers considered separately ($p=0.009$ for C1672T; $p=0.011$ for G-207C) as well as jointly ($p=0.0025$). The association was also strongly present in the perianal subgroup of CD diagnosed after 5 years disease evolution ($n=77$; $p=0.047$ for C1672T; $p=0.009$ for -207G/C; $p=0.017$ for TC haplotype). This study confirm and refine the association previously found between perianal CD and the IBD5 locus .

P1158. L-2-hydroxyglutaric aciduria: identification of novel mutations in the gene C14orf160 (duranin)

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L-2-hydroxyglutaric aciduria (L-2-HGA) is a rare neurometabolic disorder with autosomal recessive transmission (MIM 236792). It is characterized by progressive deterioration of central nervous system function including epilepsy and macrocephaly in 50 % of cases and elevated levels of L-2-hydroxyglutaric acid in urine, blood, and cerebrospinal fluid (CSF). Around 75 cases of L2-HGA have been reported from different parts of the world. We have recently reported the identification of the gene C14orf160 (duranin) for L-2-HGA using homozygosity mapping in 18 Turkish families (Topçu et al, 2004), and a second group has confirmed these results genetically and biochemically in three other families (Rzem et al, 2004).

Here we report the results of the mutation analysis in another large series of 11 additional families with different ethnic and geographic origins, from France, Germany, Iraq, Lithuania, Pakistan, Saudi Arabia and Turkey. Three of the mutations previously described in Turkish patients, were found in patients from Germany, Iraq and Turkey. We identified seven novel mutations including 4 different deletions and two different nonsense mutations; one missense mutation (R251X) was the same in patients from Turkey, France and Pakistan. The functional role of this enzyme in intermediary metabolism in humans remains to be established.

P1159. Association between CAG repeat number in the androgen receptor gene and male infertility in Macedonian males

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The exon 1 of the androgen receptor (AR) gene contains a polymorphic CAG repeat that codes for polyglutamine tract. The number of CAG repeats among normal population varies between 10-36 repeats. Several reports indicated an association of the high CAG repeat numbers and male infertility. The aim of this study was to evaluate the possible effect of long CAG repeats in the AR gene on male infertility in Macedonian men. A total of 196 infertile/subfertile males were studied. The patients were divided in six groups: azoospermia (n=70), severe oligozoospermia (n=33), moderate oligozoospermia (n=25), mild oligozoospermia (n=12), normozoospermia (n=39) and known cause of infertility, such as AZF deletions, XXY and XX males (n=17). The control group consisted of 111 proven fathers. The CAG repeat number was determined by fluorescent PCR amplification of exon 1 of AR gene followed by capillary electrophoresis on ABI PRISM 310 Genetic Analyzer. CAG repeats ranged from 13 to 32 in infertile/subfertile men and from 14 to 30 in the control group, with the most common allele of 21 in both groups. No difference in mean CAG number was found between infertile men (22.2±2.9) and proven fathers (22.3±2.9). A significantly longer CAG repeats were found only in men with mild oligozoospermia (24.6±3.0) when compared to the fertile males (p=0.022). These data indicate an association between CAG repeat length and mild oligozoospermia among Macedonian men.

P1160. Preliminary MSX1 gene analysis results in patients with cleft lip with/ without cleft palate

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BACKGROUND MSX1 is a key factor for the development of teeth and craniofacial skeleton and has been proposed to play the main role in terminal cell differentiation. Therefor non-syndromic cleft lip with/ without cleft palate (CL/P) may be very closely conjugated with changes in MSX1 gene function. Several studies show us that mutations in the gene can influence the formation of orofacial clefts. These particular mutations are causal and they appear to contribute about to 2% of all cases CL/P **OBJECTIVES** The aim of our study was to establish role of the gene MSX1 in the formation of cleft lip and/or palate. **MATERIAL AND METHODS** 50 DNA samples were collected in collaboration with Riga Stradiņi University Institute of Stomatology, State Cleft center. The collection of data was performed according to Central Ethical Committee regulations. All the participating families signed an informed consent form. We obtained questionnaire, containing detailed information: complete genealogy, possible exposure to teratogens during pregnancy, the course of pregnancy and delivery and associated anomalies. The referent gene sequence was obtained from Internet date base <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>. The methods used included DNA extraction, PCR, agarose gel electrophoresis, sequencing and blasting of the result. **RESULTS** We have found one mutation in the intron at the position 1831 where T>A, and one SNP in the second exon at the position 3969 A/G.

P1161. A common haplotype in the 5' region of the SCN5A gene is associated with ventricular conduction impairment

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Background: The SCN5A gene encodes the alpha-subunit of the cardiac voltage dependent sodium channel. Coding region mutations cause Brugada Syndrome and other familial conduction disturbances. Recent studies have suggested SCN5A promoter mutations may also contribute to arrhythmias. **Aim:** We investigated the influence of common SCN5A promoter and gene variants on ECG parameters in a central European general population sample. **Methods:** We genotyped 702 individuals from the population based KORA S2000 survey for 55 SNP markers. Haplotypes were inferred by the Haploview software package. **Results:** We identified a block of high linkage disequilibrium extending from 10 kb upstream of noncoding exon 1 to 10 kb into intron 1. Within the block the third most frequent haplotype (hap3, AF= 16.8%) was significantly associated with the width of the QRS complex (p=0.0075; QRS 93.4 ms in wt/wt (n=474), 96.3 ms in wt/hap3 (n=201) and 100.8 ms in hap3/hap3 (n=18)). The association was confirmed in the entire sample of the KORA S2000 survey (p=0.0021). It was stronger in older individuals and in those with preexisting cardiac and cardiovascular disease. These data support the concept that variability in channel expression by polymorphisms in the regulatory region of the gene influences cardiac conduction even in unselected individuals from the general population.

P1162. A high density SNP panel in the MHC region.

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The major histocompatibility complex (MHC) is a ~4 Mb gene-dense region of the human genome on chromosome 6p21. There are over 120 expressed genes in this region of which ~40% encode proteins involved in immune defense including the human leukocyte antigen (HLA) membrane glycoproteins involved in recognition of T lymphocytes. Since the classical HLA loci represent a minority of genes found in the MHC region, it is likely that many disease-causing mutations may actually reside outside one of the classical HLA genes. Therefore, since almost every autoimmune and inflammatory disorder is studied in this genomic region, we have developed a panel of approximately 3,000 SNPs that can be used as a cost-effective, efficient method for fine mapping in the region for identification of

genes associated with disease phenotypes. In addition, this panel can be used to discriminate between causal alleles and variation that is in linkage disequilibrium (LD) with causal alleles. The panel consists of two multiplexes, each with ~1,500 SNPs that are designed for use with Illumina's GoldenGate® assay and can be used either independently or in conjunction with one another. The first multiplex is "exon-centric" and contains SNPs within 10 kb of coding sequences of genes in the MHC region spanning from ret finger protein (RFP) to motilin (MLN). The second multiplex consists of SNPs evenly spaced across the region with an emphasis on tag SNPs. With these two panels combined, the average spacing between SNP loci is less than 2 kb.

P1163. Identification of the 208-2A>G "AMN" mutation in a non Jewish MGA Tunisian patient

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Megaloblastic anemia 1 (MGA1) (MIM 261100), also known as the Imerslund-Gräsbeck syndrome (I-GS) is a rare autosomal recessive condition that is characterized by a selective intestinal vitamin B12 malabsorption and proteinuria.

Currently, more than 250 MGA1 patients have been identified worldwide, but the disease was prevalent in Finland, Norway and several Eastern Mediterranean regions. I-GS is genetically heterogeneous. It has been recently shown that the disease can be caused by mutations in either the cubilin (*CUBN*) or the amnionless (*AMN*) gene.

In the present study, we investigated the molecular defect underlying IGS in 9 Tunisian patients belonging to six unrelated consanguineous families. Haplotype and linkage analyses, using the appropriate microsatellite markers surrounding both *CUBN* and *AMN* genes, demonstrated that 4 out of 6 families were likely linked to *CUBN* gene. The patients from these families have been screened for the Mediterranean already published mutations by direct sequencing of the corresponding genomic DNA regions. No one of the screened mutations was observed in our population. One family showed a linkage analysis to *AMN* gene. Direct screening for the already reported of *AMN* mutations allowed the identification of the 208-2A>G, previously described in Jewish Israeli patient from Tunisian origin. This observation suggests that the 208-2A>G mutation may derive from a single Mediterranean founder ancestor, consequence of population migration flows. For the latest family, haplotype analyses excluded both *CUBN* and *AMN* genes suggesting the presence of a third gene locus that can cause I-GS.

P1164. Cytokine gene polymorphisms and risk coronary artery disease in Tatars

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Cytokines are considered to be key players in the chronic vascular inflammatory response that is typical of atherosclerosis. Their genes are thus worthy candidates in studies of the genetic basis of coronary artery disease (CAD). We aimed to investigate whether tumor necrosis factor alpha (-308A/G TNFA), interleukin-1 beta (-511T/C IL1B, +3954C/T IL1B) and interleukin-1 receptor antagonist (IL1RA, 86-bp repeated sequence in intron 2) gene polymorphisms are associated with CAD in Tatars from Bashkortostan. Polymorphisms were detected in unrelated males without symptoms of cardiovascular diseases (127 Tatars) and CAD patients (115 Tatars) by the PCR or PCR-RFLP technique. Genotypes and allelic frequencies for these polymorphisms in both groups were compared using the Fisher's exact test. The strength of an association was expressed in the terms of relative risk, calculated as odds ratio (OR). We found genotype frequency distribution of TNFA gene in the group of patients differed from that in control group. The frequency of GG genotype was 86.96% in CAD vs. 74.80% in control (OR: 2.25, P=0.022), correspondently in these groups frequencies of AG genotype were 13.04% and 23.62% (OR: 0.49, P=0.047) and frequencies of AA genotype were 0% and 1.57% (P=0.499). Frequencies of alleles and genotypes for IL1B and IL1RA

gene polymorphisms in both groups were no significantly different. Thus our results showed that in Tatars the -308A/G TNFA gene polymorphism was associated with CAD and genetic variation in or near the TNFA locus may predispose to CAD.

P1165. Lipoprotein-associated phospholipase A2 gene (PLA2G7) and multiple sclerosis in Italian patients

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Several studies suggest that a pre-existing Lp-PLA2 deficiency may be a risk factor for several diseases having an inflammatory component such as multiple sclerosis (MS). We have evaluated the association of PLA2G7 gene polymorphisms with susceptibility to MS in a study including 95 MS patients and 114 healthy controls. A total of six single nucleotide polymorphisms (SNP) throughout the gene were examined: three missense polymorphisms (Arg92His, Iso198Thr, and Val379Ala), a deletion polymorphism (1190-20_23delGATT) in intron 11, and two polymorphisms (-402T>C and -209C>G) in the 5'-flanking region. Single SNP frequency analysis showed a significant association of the SNP -209C>G with MS (p=0.003; OR= 2.4, CI:1.29-4.5. Fisher exact test). Haplotype reconstruction revealed seven common haplotypes accounting for >80% of the chromosomes observed. Logistic regression analysis showed the association of a haplotype containing the allele -209G with MS (p=0.018 ; OR: 2.48, CI: 1.58-3.58). No other haplotype revealed any significant association. This preliminary result suggests an association of -209C>G SNP with increased susceptibility to MS. The genotyping of a larger set of individuals may indicate possible interaction effects among the SNPs.

P1166. Linkage analysis in a large nonspecific X-linked Mental Retardation (MRX) family.

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X-linked mental retardation (XLMR) is a clinically heterogeneous set of conditions responsible for a large proportion of inherited mental retardation. XLMR conditions were subdivided into specific (MRXS) and nonspecific (MRX) forms, depending on their clinical presentation. MRX families, whose only phenotypic manifestation is mental delay, can only be distinguished by their positional mapping, eventually leading to gene identification. We here report on linkage analysis in a large family including 4 affected males with MRX and 4 obligate carrier females. Linkage analysis using 30 polymorphic markers spanning the entire X chromosome defined a candidate region between DXS1003 (Xp11.3) and DXS1216 (Xq13.1). LOD scores are significant (>2). We also studied the X inactivation pattern in this family with the methylation test of the AR gene. We found skewage of X inactivation in an obligate carrier, her carrier daughter and in a sister whose carrier status is uncertain. Her carrier aunt, mother of an affected individual, showed random X inactivation. This latter finding suggests that skewage of X inactivation is not always an indicator of carrier status in XLMR families. We are currently screening candidate genes in the approximately 20 Mb region delimited by DXS1003 and DXS1216, including 6 genes whose mutations have already been described in other MRX families.

P1167. Association study of Estrogen receptor-alpha gene with mineral bone density in a group of unrelated Italian females

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Estrogen receptor-alpha (ESR1) gene variants have been widely investigated for their putative role in influencing bone mineral density. In a recent multipoint analysis with variance component that we performed on 118 Italian families recruited through an osteopenic/osteoporotic member, we found a weak linkage with ESR1 Pvull/XbaI haplotype at

three densitometric sites. In a family based association study (TDT), we found that the T/A-Pvull/XbaI haplotype was preferentially non transmitted to affected sibs ($p<0.01$). An association was observed between C/G or C/A haplotypes with low BMD at spine and femur (Sangalli et al., submitted). We decided therefore to investigate further a putative association of ESR1Pvull/XbaI haplotypes with BMD in a sample of 182 peri and postmenopausal Italian women. Association analysis of the single markers with BMD values at spine and femur did not show any significant association. Haplotypes were reconstructed and no significant association with BMD values at the 2 distinct sites was observed. Genotyping has been extended to a larger set of females.

P1168. Genetic variability of mu-opioid receptor and anaesthesiological implication: preliminary study

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There is interindividual variability in pain perception and sensitivity to analgesic agents. The opioids drugs are widely used in management of perioperative pain, but individual differences in the effectiveness and adverse effects of these drugs have been described. The μ receptor, encoded by OPRM1 locus, is the major target for endogenous and exogenous opioids. OPRM1 is a candidate gene for pain tolerance and differences in individual responses to opioids.

The most frequent SNP, about 10-20% is a adenine substitution to guanine at position 118 resulting in aminoacid change at position 40 of aspartate into asparagine (ND40). In vitro, ND40 substitution displays three-fold enhanced binding of beta-endorphin that common allel. SNPs have not been studied in relation to intraoperative and postoperative response. 18 healthy women who gave birth undergoing elective cesarean section under epidural anesthesia were randomized to receive either Levobupivacaine alone or levob. plus Sufentanil. Basal conditions were assessed on T0, every 5 minutes until T45min, on T6h and T24h, with measurement of physiological parameters. The post-operative pain was estimated on T6h and T24h by means of scale VAS (visual analog scale). IL-6 and IL-10 measurement were performed on T0, T45min, T6h and T24h blood samples. The coding and flanking intron region of μ receptor gene was screened using DHPLC method. In our cohort we found 5 heterozygous patients for N40D change. Ours preliminary results suggest a possible correlation between ND40 change and pain control: lower increase of anti-inflammatory cytokines and lower VAS value are present in heterozygous subjects.

P1169. Coagulation factor polymorphisms in women with recurrent spontaneous abortion (RSA)

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Maternal coagulation factor mutations and gene polymorphisms have been associated with adverse pregnancy outcome. We conducted a case-control study to determine the association between the G20210A prothrombin and G1691A factor V point mutations, the factor VII Arg353Gln and factor XIII Tyr204Phe gene polymorphisms and the risk of RSA.

Of a total of 54 Caucasian women with a history of three or more consecutive spontaneous abortions before the 20th week of gestation, 51 of them attended the Division of Medical Genetics, University of Medical Center Ljubljana and three of them were ascertained at the Department of Medical Biology and Genetics, Faculty of Medicine Comenius University Bratislava. As a control group, 55 age and ethnicity matched controls with at least two live births and no history of pregnancy loss were included. Polymerase chain reactions were performed to analyze the point mutations of G20210A prothrombin and G1691A factor V, and the gene polymorphisms of factor VII Arg353Gln and factor XIII Tyr204Phe.

We found statistically significant differences in factor VII gene

polymorphism frequencies. The frequency of factor VII Arg353Gln heterozygotes in the control group was 29% (16/55) versus 11% (6/54) in the study group ($p=0.019$; OR=3.28; 95% CI=1.17-9.18). No significant differences in frequencies of genotypes of other tested coagulation factors and the occurrence of RSA were observed.

Our findings implicate that Arg353 allele of coagulation factor VII might be a protection factor against RSA. Further studies comprising of larger numbers of women with RSA are needed to confirm our findings.

P1170. First evidence that a rare genetic polymorphism with high penetrance may be involved in the aetiology of endometriosis

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Background - Endometriosis (the presence of endometrial-like deposits outside the uterus) is a common complex disease associated with pelvic pain and subfertility in women of reproductive age. Its aetiology remains largely unknown, although familial aggregation of the condition has been demonstrated in humans as well as non-human primates.

Methods - In a recently conducted genome-wide linkage study, 256 Caucasian families each containing at least 2 sister-pairs with endometriosis were recruited; 52 of these families were found to contain 3 or more affected members (41 families with 3 cases; 10 with 4; and 1 with 5). Non-parametric genome-wide linkage analyses in the 52 families were conducted in Merlin, using sex-specific recombination information from the Rutgers linkage-physical map. Parametric linkage analyses were conducted using GeneHunter.

Results - Six LOD score peaks with Kong & Cox LOD scores greater than 1 were found. One of the peaks, on chromosome 7, reached a LOD of 3.49 (simulation-based genome-wide significance value: $p=0.007$). Subsequent parametric analyses provided a heterogeneity LOD of 3.92, using a model of a low frequency (0.002), highly penetrant (0.85 for heterozygotes; 1 for homozygotes) disease allele. Results of haplotype analyses using additional markers in the region, and of further phenotypic analyses exploring the presence of subfertility and pelvic pain, will be presented.

Conclusion - A rare genetic polymorphism with high penetrance may be involved in the aetiology in a subgroup of women with endometriosis. Finding this polymorphism and its function may provide a first insight in the pathologic pathway of this condition.

P1171. Evaluation of Nyholt's Procedure for Multiple Testing Correction

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A simple method for accounting efficiently for multiple testing of many SNPs in an association study was recently proposed by Nyholt (2004), but its performance was not extensively evaluated. The method involves estimating an 'effective number' of independent tests and then adjusting the smallest observed p-value using Sidak's formula based on this number of tests. We sought to carry out an empirical and theoretical evaluation of Nyholt's method.

Nyholt's method was applied to a sample of 31 genes typed at a total of 291 SNPs and permutation used to determine the type-I error rate for each gene.

The nominal 5% type-I error rate varied from under 3% to over 7%, and was dependent on linkage disequilibrium. Theoretical considerations show further that the method can be very conservative in the presence of haplotype block structure.

Although Nyholt's approach may be useful as an exploratory tool, it is not an adequate substitute for permutation tests.

P1172. Familial secundum atrial septal defect maps to chromosome 15

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Atrial septal defect (ASD) is a common congenital heart defect affecting 4 in 100,000 individuals. ASD of the secundum type is characterised by an incomplete coverage of the ostium secundum resulting in a left-to-right shunting. The effects of the ASD range from subclinical to severe symptoms including pulmonary hypertension, arrhythmias and heart failure. ASD is mostly sporadic but several familial cases have been reported. We describe two extended Swedish families with isolated autosomal dominant ASD. Using polymorphic microsatellite markers, we performed a genome wide scan in search for chromosomal regions linked to ASD. Linkage to the previously described candidate loci including the *COMP* and *GATA4* genes were excluded for the two families. We identified a 23 cM region on chromosome 15 (15p11-q13) which is linked to the disease phenotype. The region is restricted by recombination events between the markers D15S165 and D15S659 and we calculated a maximum cumulative LOD score for the two families of 3.6 assuming full penetrance. Candidate genes are now investigated in the linked region for structural mutations from affected family members.

P1173. Analysis of influence of genes involved in control of bone and cartilaginous metabolism on pathogenesis of idiopathic osteoarthritis of knee-joint

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We studied an association of polymorphic allelic variants of genes, involved in control of bone and cartilaginous metabolism with clinical course and severity of idiopathic osteoarthritis of knee-joint (IOKJ). Study group included 112 patients with idiopathic osteoarthritis of knee-joint. Patients were diagnosed according to clinical and radiographic data. According to disease anamnesis, intensity of functional abnormalities, efficiency of conservative methods of treatment and successfulness of clinical effect patients were divided onto three groups with different kind of IOKJ progression: slowly, moderately and rapidly progressive. Also all patients were subdivided onto three groups by stages of degenerative-dystrophic lesion of knee-joint according to clinical and radiographic classification by Kossinskaya. The comparison of genotypes frequencies of the vitamin D3 receptor (VDR), collagen type I (COL1A1), collagen type II (COL2A1), calcitonin receptor (CALCR) and osteocalcin (BGLAP) genes in patients with IOKJ and in controls revealed an association between the TT genotype of the CALCR gene and rapidly progressive osteoarthritis of knee-joint and also an association of the hh genotype of the BGLAP gene with mild IOKJ.

Conducted analysis of genotypes association of the VDR, COL1A1, CALCR and BGLAP genes taking into account the interaction of alleles of these genes allowed to reveal an association of the TT Tt genotype (CALCR and VDR) with rapidly progressive OA and the hh SS genotype (BGLAP and COL1A1) with severity of disorder. Thus all the genes we have studied which control bone and cartilaginous metabolism directly or indirectly involved in pathogenesis of osteoarthritis on different stages of disease.

P1174. The MTHFR 677C>T/1298A>C double heterozygous genotype CT/AC occurred in higher frequency in the older group of abdominal aortic aneurysm patients

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In a group of 83 males with abdominal aortic aneurysm (AAA), treated with trans-abdominal surgery, the two polymorphisms of the MTHFR gene (677C>T and 1298A>C) were determined. The group has been characterized for the diabetes mellitus (DM) co-occurrence and age of the aneurysm development to diameter of more than 50 mm (at which the surgery was performed). The age range was 51-81; DM was diagnosed in 15 patients. The group of non-DM AAA patients

was divided according to the age of surgery into the <=65 and >65 years old. In the older group (n=43) the frequency of MTHFR 1298 AC genotype (59%) was higher as compared to that in the younger (n=25) group (34%) (p=0,02, Fischer exact test). Also the frequency of the MTHFR 677C>T/1298A>C combined heterozygotes (CT/AC) was higher in the older group of non-DM AAA patients (36%) as compared to that in the younger group (17%) (p=0,04, Fischer exact test). The frequency of MTHFR 677 CT genotype in the older group (46%) did not differ from that in the younger group (45%). We conclude that the age of the aneurysm development as well as the DM co-occurrence may influence the assessment of the role of the the MTHFR gene polymorphisms in pathogenesis of AAA. The finding of the increased frequency of the MTHFR 677C>T/1298A>C double heterozygous genotype CT/AC in the older patients group expands also the earlier finding of the association between the AAA occurrence and the MTHFR 677CT genotype (Strauss, 2003).

P1175. Plasminogen Activator Inhibitor-1 4G/5G polymorphism and cardiovascular disease in Iran

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Plasminogen activator inhibitor-1 (PAI-1) molecule is primarily known for its role in counteracting plasminogen activation and impairing fibrinolysis. It has been observed that high plasma PAI-1 levels are associated with an aggravated prognosis of myocardial infarction in the acute phase. On these bases, PAI-1 has been implicated in thrombotic events and has been considered as a risk factor for cardiovascular disease. PAI-1 also plays a role in several pathophysiological processes, presumably independent of plasminogen activation: The link between PAI-1 and the metabolic syndrome has been long established. During the last decade, strong association has emerged between plasma PAI-1 levels and parameters of the metabolic syndrome (Body Mass Index, visceral fat, blood pressure, plasma levels of insulin or proinsulin, triglycerides, LDL particles, free fatty acids, and HDL cholesterol). One line of research has focused on genetic polymorphisms of PAI-1, particularly the 4G/5G insertion/deletion in the promoter region of the gene. This polymorphism affects transcription rates. 4G/5G polymorphism has been investigated in connection with cardiovascular disease in association studies in various populations with varying results. Here, we report preliminary results of our ongoing case-control association study in Iran, which point to a possible link between PAI-1 4G/5G polymorphism and cardiovascular disease in this population.

P1176. Haplotype analysis of tumor necrosis factor alpha / lymphotxin alpha, interleukin 1, -10, and vitamin D binding protein in COPD patients from Russia

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Inflammation plays an important role in the development of chronic obstructive pulmonary disease (COPD). It has been suggested that the inflammatory mediators' imbalance play a role in chronic inflammation. We investigated a panel of polymorphisms: interleukin 1b(-511C/T and +3953C/T) and the IL-1 receptor antagonist (VNTR 86 bp in intron 2), tumor necrosis alpha (-308G/A) and lymphotxin alpha (252A/G), interleukin 10 (-592A/C and -627C/A) and vitamin D binding protein (416Glu/Asp and 420Thr/Lys in exon 11) genes in patients with COPD to determine whether haplotypes of these genes are linked to a genetic susceptibility to COPD.

Gene polymorphisms were examined in 282 individuals affected COPD and compared with a healthy control group of 227 subjects. Polymorphisms were examined by a PCR-RFLP method. Haplotype frequencies were estimated with EH software program (Rockefeller University; NY).

The likelihood ratio test showed a significant linkage between the

alleles of the TNF/LTA gene family, IL1 and IL10 polymorphisms both in patients and controls. For IL10 haplotypes differences in frequencies between cases and controls reach significance ($x^2=18.58$, $p<0.001$). The frequency of -592C/-627A haplotype was higher in COPD patients (21% versus 14% in controls, $x^2=6.39$, $p=0.01$, OR=1.6, 95%CI 1.10-2.33), thus, another haplotype -592C/-627C was significantly decreased (13% in controls versus 6% in COPD). In case of VDBP, statistically powered linkage between exon 11 polymorphisms was observed only in COPD patients group.

Our results provide new insights on a possible involvement of IL10 gene in susceptibility for COPD even though further investigations are necessary to clarify findings.

P1177. The G2019S LRRK2 mutation in autosomal dominant European and North African Parkinson's disease is frequent and its penetrance is age-dependent

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Parkinson's disease (PD), the second most common degenerative disorder of adulthood, is characterized clinically by rigidity, bradykinesia, resting tremor, and postural instability with good initial response to levodopa.

Mutations in LRRK2 (PARK8) were recently identified in autosomal dominant Parkinson's disease (ADPD), including the G2019S mutation that was found in different populations. In order to evaluate its frequency, we analyzed 200 index cases with ADPD, mostly from Europe and North Africa. Surprisingly, the frequency in North African families (41%; CI 18.4-67.1) was much greater than in those from Europe (2.8%; CI 0.9-6.5) ($p<0.001$). The clinical features in 21 patients, including one with an homozygous mutation were those of typical PD but with later onset and lower Mini Mental scores (MMSE). There were also 15 still unaffected mutation carriers, aged 32 to 74, indicating the existence of age-dependent penetrance. LRRK2 mutations appear to be a common cause of ADPD, particularly in North Africa.

P1178. CF modulating genes

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Cystic fibrosis (CF) is a lethal genetic disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. Pulmonary disease in CF patients, the main cause of morbidity and mortality of these patients, is variable and influenced by secondary genetic factors as well as environmental factors. Individual genes were initially investigated in a considerable number of studies for a modulating effect on the CF phenotype. More lately, larger association studies have been set up aiming at the study of many genes for their potential modulating effect on the CF phenotype. We performed a study in Belgian and Czech CF patients, aiming at the analysis of 51 genetic variants from 22 genes. We focused on proteins that are part of the host defense/protection system and proteins that directly or indirectly interact with CFTR. It was found that genetic variants from seven genes involved in the host defense/protection system and two genes encoding proteins that interact with CFTR influence CF pulmonary function, indicating that a more efficient immune system is beneficial to CF patients. However, when looking at all CF association studies performed for any gene, a 100% consensus of a gene being a CF modulator or not, is not obtained. Most studies use different designs,

and although the study-design of some studies may be questionable and therefore result in contradictory wrong findings, the findings of the different studies, especially the larger ones, will be complementary and their findings should be interpreted in that context. An overview of the findings, limitations and recommendations of CF association studies will be given.

P1179. Molecular analysis of eotaxin gene SNPs in Italian children with atopic dermatitis

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Atopic dermatitis (AD) is often associated with a family history of atopy. Two types of AD have been identified: the extrinsic (EAD), or allergic form, and the intrinsic (IAD), or non allergic form.. In our study, the prevalence of genotypes at -426 C>T,

-384 A>G and 67 G>A single nucleotide polymorphism (SNP) sites of eotaxin gene have been investigated in EAD and IAD children and in their relatives. Seventy and seven children (44 females and 43 males) (age range 3 months-12 years) suffering with AD were selected from 31 nuclear families (Department of Paediatrics, University of Messina, Sicily, Italy). The severity of AD was measured through the SCORAD Index. Genotyping was carried out by the PCR-RFLP method. We amplified three regions of the eotaxin gene, each of which included a SNP site,

-426C>T, -384A>G, 67G>A. As regard the -426 SNP genotype, a significant difference was found between EAD children ($p<0.05$) and EAD relatives, and nonatopic control ($p<0.05$); as well between EAD and IAD children ($P<0.01$).The frequency of this SNP was no different between the IAD relatives and the control group. No significant association was observed between the 67G>A and -384A>G SNPs and EAD children in respect to control group; as well between IAD children and controls. Similar results have been found in their relatives. Our data underline the possible role of some specific SNPs of the eotaxin gene in the development of extrinsic atopic dermatitis in the children with a familial occurrence of AD and/or other allergic diseases.

P1180. Analysis of genetic polymorphisms in élite Sardinian (Italy) athletes.

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In human endurance performance capacity is characterized by a large degree of inter-individual variation in the general population and even in well-trained, athletic individuals. There is a little doubt that performance is influenced by environment, as the effects of diet and training on athletic ability have long been known, if not completely understood; however, the contribution of an individual's genetic make up is less clear. The dominance of particular nationalities, ethnic groups, or families in various sporting events is often perceived as evidence that heritage (biological or cultural), plays a role in the development of athletic skills. Numerous researchers have attempted to elucidate the effects of genetic background on physical performance and to identify the specific genetic variants that contribute to performance. In order to contribute to estimate the importance of the genetic on endurance performance capacity, we used molecular biology and genetic advanced methods through the study of polymorphic genes in élite athletes. The examined polymorphisms have a correlation with the physiological parameters that are involved in endurance performance capacity. 37 élite Sardinians (Italy) athletes (10 males and 27 females) were studied. They practised hockey, soccer, rhythmic gymnastic, artistic gymnastic, and track athletic. The samples were analyzed for one Alu polymorphism (ACE) and six RFLP markers (APOE, FGB HindIII, FGA Taql, FBG BclI, PAI HindIII, GplIIa Taql). The results were compared with a control samples from Centre and South Sardinia. Preliminary results showed a relevant significant correlation between élite athletes and ACE and GplIIa genotypes.

P1181. Interleukin 1 Alpha Gene 5'- Regulatory Region Polymorphism in Alzheimer's Disease

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Interleukin-1 is a pluripotent immunomodulatory cytokine that has an initiating role in cellular and humoral immunity in the periphery. It is reported that a polymorphism in the 5'-flanking regulatory region at -889 of the interleukin-1 alpha (IL-1alpha) gene may cause an over expression of IL-1 alpha, which is also shown to be associated with inflammatory diseases and Alzheimer's disease. In this preliminary study, our aim was to determine if there is an association between IL-1 alpha gene and late-onset Alzheimer's disease. We collected blood samples from 52 cases of dementia of Alzheimer type and from 35 age-matched controls (mean ages 75.1±5.7, and 72.7±7.3, years respectively). Patients are clinically diagnosed by Istanbul University, Cerrahpasa Faculty of Medicine, Department of Geropsychiatry and Istanbul University, Istanbul Faculty of Medicine, Department of Neurology, Behavioral and Movement Disorders Unit according to DSM-IV criterias. Salting-out method with 5M NaCl is used for DNA isolation. We used polymerase chain reaction-confronting two-pair primers (PCR-CTPP) to test for an association between Alzheimer's disease and a polymorphism at -889 of the IL-1 alpha gene. After genetic analysis of the IL-1 alpha gene, we found 63.5% genotype C/C, 32.7% genotype C/T, 3.8% genotype T/T for patients, and 48.6% genotype C/C, 45.7% genotype C/T, 5.7% genotype T/T for healthy control. When the control and patients were compared for C/C, C/T and T/T genotypes we saw that the distribution of genotypes and alleles did not differ according to Chi-square test ($p=0.39$). Our preliminary results show no significant increase in the risk for the T/T or C/T genotype in late-onset cases. Thus, we were unable to find an association between the C -889T transition on IL-1 alpha gene and late-onset Alzheimer's disease.

P1182. A Comparison of Disease-Based linkage disequilibrium in HLA haplotypes of multiple sclerosis and narcolepsy patients: A novel approach for dissecting HLA associations

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Multiple sclerosis (MS) and narcolepsy are distinct neurological disorders, which are both associated with the HLA-DRB1*1501-DQB1*0602 (DR15) haplotype. For MS, however, strong evidence exists for another risk locus at the telomeric end of HLA complex. Thirteen microsatellite markers (D6S2236-G51152), and the DRB1/DQB1 genes, were genotyped in 166 MS simplex families from the Australian State of Tasmania, and 153 Narcolepsy simplex families from the USA. Allelic association was observed for three microsatellite markers: MOGCA ($p=0.002$), HLA-FCA ($p=0.03$) and D6S265 ($p=0.05$) at the telomeric end of the classical class I region only in MS families. We compared haplotype-specific linkage disequilibrium (LD) for the extended 7.1 (HLA-A*0301-B*0702-DR15) haplotype in MS and narcolepsy patients to map the telomeric MS risk locus. Haplotypes were inferred using MERLIN, and a novel algorithm (HAPLOCLUSTERS) was used to determine haplotype association across 6.79 Mb of the HLA complex. Long-range haplotype association was observed in MS families across the entire region, and association was strongest ($p<0.00001$) from MOGCA to G51152. Telomeric of MOGCA, association decreased gradually across the extended class I region, and D6S105 was identified as the distal boundary of the disease risk interval. In narcolepsy families, haplotype association was confined to a genomic segment centromeric of D6S265 and distal of G51152. There are a number of pathologically distinct common human

diseases, which are associated with the same HLA variant, or ancestral HLA haplotype. We propose that our approach to dissecting population LD from disease-based association should be broadly applicable for the genetic mapping of other HLA loci.

P1183. Genetic predisposition to athletic performance: analysis of ACTN3 R577X polymorphism by real time pcr.

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Athletic performance is influenced by training, environmental factors and genetic predisposition. Independently by the individual attitude and skills, muscle and sarcomer proteins represent the fundamant of an effective movement. Alpha-actinins (ACTN) are a family of actin-binding proteins related to dystrophin. A premature stop codon in ACTN3 gene (11q13-14) in position 1.747 on exon 16 (R577X) has been shown to play a role in sport performance, even though it seems not involved in human myopathies. ACTN3 is present only in type II fibres and congenital deficiency involves about the 16% of the world population, suggesting that its absence at the Z lines of scheletal muscle fast fibres can be compensated by other factors.

We have developed a real time pcr procedure to simultaneously type R577X and Q523R polymorphisms and evaluated their distribution in the general population (n= 102) and in a sample of athletes. Data were compared with previous studies and confirmed the presence of a large percentage of homozygote subjects deficient in ACTN3 in the general population. In this Italian population sample we observed the following R577X polymorphism distribution: 22% XX homozygote, 47% RX heterozygote, 31% RR homozygote. Allelic frequencies did not significantly differ in the endurance athletes even if variations were observed. Haplotype distribution confirmed the presence of an interesting linkage disequilibrium. Analysis of polymorphisms at sarcomeric proteins opens up promising perspectives not only for understanding the rehabilitation process in myopathies, but also for shedding light on the role of genes in sport excellence predisposition.

P1184. Angiotensin I-Converting Enzyme (ACE) and Low-density lipoprotein Receptor-related Protein-Associated Protein 1 (LRPAP1) gene polymorphisms in a population of northern Italy

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In light of the significant impact of the Angiotensin I-Converting Enzyme (ACE) and Low-density lipoprotein Receptor-related Protein-Associated Protein 1 (LRPAP1) gene polymorphisms on genetic susceptibility to cardiovascular diseases, the present work attempts to determine the frequencies of ACE and LRPAP1 allele polymorphisms in a control populations of Nord-West Italy (525 unrelated individual aged between 1 and 100 years, mean age 48±22.2 years). In order to analyze the relationship between ACE and LRPAP1 gene polymorphisms and age, the sample was subdivided in 3 age groups: 1-30 (n = 146); 31-50 (n = 199) and 51-100 years old (n = 117).

ACE genotype frequencies were: 6,10% for *Alu* insertion genotype (I/I), 38,10% for genotype without *Alu* insertion (D/D) and 55,80% for I/D genotype. Allele frequencies were 43,52% for the I allele and 56,48% for the D allele. Differences in D/D genotype frequency between age groups were significant, with the lower value in the more aged group (χ^2 test, $0.01 < p < 0.02$). This result could suggest a selective advantage for I/I genotype. In the case of LRPAP1 gene, the genotype frequencies were: 1,15% for the normal genotype (I/I), 63,79% for the 25 bp double deleted genotype (D/D) and 35,06% for the I/D genotype. Allele frequencies were 26,31% for the I allele and 73,69% for the D allele. No sex differences were found. For LRPAP1 gene the more aged group showed a significant higher value in D/D genotype frequency ($0.001 < p < 0.01$). No significant differences between groups were found in ACE I/I-LRPAP1P1 D/D double genotype frequency.

P1185. Cystic Fibrosis is not a frequent disease in Cyprus except for a village with founder effect and very high carrier frequency of 1/14

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Cystic Fibrosis is a common autosomal recessive condition in Caucasians, with average carrier frequency of 1/25. In the Greek-Cypriot population of about 650000 we diagnosed during the past 12 years, 27 patients, 24 of whom are alive. Thus, about 1/83 people is a carrier of a CF mutation. The most frequent mutation is F508del, accounting for 43.8% of mutant alleles. The second most frequent is L346P (12.5%), carried by 6 patients in heterozygosity. Apparently this is a Cypriot mutation, as it has not been reported in other populations, to our knowledge. It is found in compound heterozygosity with other severe mutations, and we reckon it is a mild mutation dominating over the severe one, since all patients carrying it have milder phenotypes. Other mutations are: 1677delTA (6.2%), 3849+10kbC>T, (4.2%), S549N (4.2%), N1303K (4.2%), M348K (2.1%), 2789+5G>A (2.1%), W1282X (4.2%), G542X (2.1%), Unknown (12.5%). Interestingly, in Athienou, a village of 4500 people, we located a large family with two affected siblings, presently deceased. In an epidemiological study involving more than 800 people, 1/14 was a carrier of F508del, most probably as a result of founder effect, dating back to the times that the Ottomans conquered Cyprus. The legend has it that after the Ottomans conquered Famagusta at the Southeast coast, some noble Franks who escaped the massacre, moved to a region close to Athienou. Interestingly, most of our patients, >70%, presented with dehydration and electrolytic disturbance during summer months. During the past years we performed 8 prenatal diagnoses.

P1186. Y-chromosome bi-allelic and STR markers in the three main ethnic groups of modern Bosnia-Herzegovina

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The variation at 28 Y-chromosome bi-allelic markers was analysed in 256 males (90 Croats, 81 Serbs and 85 Bosniacs-Muslims) from Bosnia-Herzegovina. In addition, 100 individuals (35 Bosniacs, 31 Serbs, 34 Croats) have been also analyzed at twelve Y-chromosomal short tandem repeats loci. An important shared feature between the three ethnic groups is the high frequency of the "Palaeolithic" European-specific haplogroup (Hg) I, a likely signature of a Balkan population re-expansion after the Last Glacial Maximum. This haplogroup is almost completely represented by the sub-haplogroup I-P37 whose frequency is higher in the Croats (~71%) than in Bosniacs (~44%) and Serbs (~31%). Other rather frequent haplogroups are E (~15%) and J (~7%), together with R-M17 (~14%). Hg E, almost exclusively represented by its subclade E-M78, is more common in the Serbs than in Bosniacs and Croats; Hg J observed in only one Croat, encompasses ~9% of the Serbs and ~12% of the Bosniacs, where, it shows its highest differentiation. Differently, Hg R-M17 harbours similar frequencies in all three groups. Overall, the three main Bosnia-Herzegovina groups, in spite of some quantitative differences, share a large fraction of the same ancient gene pool. At the STR analyses, 81 different Y-STR haplotypes were detected, 69 of them were unique. Six of twelve not singleton haplotypes were shared by different population groups: two of them by Croats and Bosniacs, two by Bosniacs and Serbs and the last two by Serbs and Croats, thus testifying recent gene flows between groups.

P1187. The epidemiology of Leber hereditary optic neuropathy (LHON) in Finland

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Leber hereditary optic neuropathy (LHON) has been estimated to be one of the most common mitochondrial disorders, with a prevalence of 3.22 of 100 000. In Finland, among a population of 5.2 million individuals, we have identified 36 genealogically unrelated LHON families with at least one affected family member. During the 25-year research period, we have identified large families, collecting information mainly by going through Finnish parish records. The three primary mutations in the mitochondrial DNA (mtDNA) and mtDNA haplogroups were determined in all families; in addition, entire mitochondrial genomes were sequenced in some patients. Heteroplasmy was detected in 14% (5/36) of the families. Penetrance values were determined for all pedigrees separately and combined; and for instance, for different primary mutations and genders.

The total penetrance of LHON was notably higher among males (32%) than among females (10%). The male-female ratio was 3.2:1 which is in accordance with previous studies. A combined penetrance value calculated for all families varied only slightly between different primary mutations.

P1188. São Miguel: the genetic history of an Azorean island described by HLA Class I and Class II genes

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São Miguel is the biggest and most populated island (131,609 inhabitants) of the Azores archipelago (Portugal). Here, we present the frequencies of alleles and haplotypes for Class I (A, B and Cw) and II (DRB1, DQB1, DPA1 and DPB1) at the DNA level in São Miguel. Blood samples were taken, after informed consent, from 106 unrelated blood donors, whose parents were born in the same locality. HLA typing was carried out using PCR-SSP from Olerup SSP. Statistical analysis was performed with Arlequin v.2.0. and phylogenetic trees were constructed by using the genetic distances between populations (DA) with the DISPAN software. Our data shows that the gene diversity at the seven loci is high (0.9997) and that all genotype frequencies are in Hardy-Weinberg equilibrium. We identified 16 HLA-A alleles, 24 HLA-B alleles, 13 HLA-C alleles, 6 HLA-DPA1 alleles, 22 HLA-DPB1 alleles, 5 HLA-DQB1 alleles and 13 HLA-DRB1 alleles, of which the most frequent are, respectively, A*02 (GF=0.2500), B*44 (GF=0.1557), Cw*07 (GF=0.3113), DPA1*01 (GF=0.4623), DPB1*0401 (GF=0.3161), DQB1*03 (GF=0.3208) and DRB1*07 (GF=0.1698). The most frequent three-locus haplotype in S. Miguel is of West European origin, HLA-A*01-B*08-DRB1*03 (HF=0.0802). We also found the Iberian-North African haplotype HLA-A*30-B*18-DRB1*03 (HF=0.0047), the Iberian-Berbers haplotype HLA-A*02-B*51-DRB1*13 (HF=0.0047) and a Mongol haplotype HLA-A*02-B*44-DRB1*04 (HF=0.0142). These observations suggest an important mixture of alleles from geographically distinct areas, which agree with the history of settlement and molecular data from Y-chromosome. The HLA data presented here will be useful to study the molecular basis of autoimmune diseases in Azores. (paularpacheco@hdes.pt) Funded by DRCT-Azores.

P1189. Demographic and baseline clinical data of 638 patients with Fabry disease: latest analysis from FOS - the Fabry Outcome Survey

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Background: Fabry disease is an X-linked lysosomal storage disorder caused by deficient activity of the lysosomal enzyme α -galactosidase A. Progressive accumulation of the enzyme substrate in cells throughout the body leads to organ failure and premature death. Treatment for this disease has recently become available in the form of enzyme replacement therapy (ERT). To determine the efficacy and

safety of ERT with agalsidase alfa, FOS - the Fabry Outcome Survey - was established. The present analysis of the FOS database aims to provide demographic information and baseline data on the natural history of Fabry disease.

Methods: The FOS database was analysed in terms of patient demography and baseline manifestations of Fabry disease.

Results: As of November 2004, 638 patients (387 receiving agalsidase alfa and 251 currently untreated) were enrolled in FOS from 11 European countries. For the first time since FOS was initiated in 2001, there are more female patients (n=324) than males (n=314); 59 of the female and 48 of the male patients are under 18 years of age. Approximately one-third of these children have significant signs and symptoms before 10 years of age. A severity score, based on the Mainz Severity Score Index, confirms the progressive nature of Fabry disease with age in both male ($r=0.53$; $p<0.001$) and female ($r=0.48$; $p<0.001$) patients.

Conclusion: FOS is providing valuable information on Fabry disease not only in men, but now also in women and children. The baseline data available will allow the effects of ERT with agalsidase alfa to be quantified.

P1190. Genetic diversity of the Azorean population revealed by 13 short tandem repeats

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The knowledge of population history, demography and genetic structure has proven to be fundamental to address research in human genetics. Here, we describe the genetic diversity of Azorean population and its affinity with other populations by the analysis of 13 microsatellite loci (TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S539, D18S51 and D21S11) in 257 unrelated blood donors. These short tandem repeat (STR) markers were typed by Polymerase Chain Reaction (PCR) with fluorescently labelled primers. Allelic frequencies were statistically analysed using Arlequin v.2.0, and phylogenetic trees based on Nei's genetic distance were constructed with the DISPAN software.

Analysis of microsatellite loci shows that the Azorean population presents a very high gene diversity (1.000). For each marker, gene diversity range between 0.661 for TPOX and 0.8812 for D18S51. Heterozygosity values calculated for each STR varies from 64.2% for TPOX to 87.1% for D18S51, although the majority of markers show values superior to 80%. Moreover, allele frequencies are similar to those obtained for mainland Portugal and other European populations. This result is corroborated by the dendrogram, in which Azores clusters with Italians, Belgians, Portuguese and Spanish, apart from Moroccans and Cabo Verdeans.

Taken together, these data indicate that the gene pool of the Azorean population is very diverse and are consistent with our previous results on Y-chromosome (Pacheco PR, Ann Hum Genet, 2005). Moreover, the major affinity with Portuguese and other European populations is primarily guided by the settlement history of the Azores archipelago. (claudiacbranco@hdes.pt). Funded by DRCT (Azores).

P1191. The DNA bank from the population of São Miguel Island (Azores): a resource for genetic diversity studies

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The peopling of São Miguel Island in the 15th century was made by Portuguese and settlers of foreign origin (Flemish, Jews, Moorish prisoners and black slaves). There is a signature of admixture in the settlement history of São Miguel, thus to unravel the population genetic background, we decided to establish a human DNA bank in order to characterize the population's polymorphisms.

Here, we describe the construction of the DNA bank, and analyse the information of 1000 samples obtained from healthy blood donors. The bank follows the international ethical guidelines, which include

Informed Consent, confidentiality, anonymity of personal data, and abandonment in case of expressed will. DNA was isolated from blood samples, coded and immediately stored in a locked refrigerator. The identifiable DNA bank has self-reported data concerning sex, age, birth, current place of living, and parental birthplaces. The samples are representative of all the island's municipalities ($r=0.995$, $p<0.01$). The majority (87%) of the participants are male, with mean age of 36.3 y (18-64). Birthplace analysis reveals that 902 (90%) have both parents born in São Miguel. Moreover, 477 (54%) have their parents born in the same locality, confirming high rate of consanguinity in rural area. To date, this DNA bank was used to assess the Y-chromosome phylogeny and diversity in Azorean population (Pacheco PR, Ann Hum Genet, 2005, in press). Now, we are analysing autosomal STRs for the better understanding of the gene pool and genetic structure of the archipelago's population. (lmotavieira@hdes.pt). Funded by DRCT, Azores.

P1192. Haplotype distribution and Polymorphism in the non-coding region of human Mitochondrial DNA in persian patients harboring LHON primary G11778A, G3460A, T14484C and G14459A mutations

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Leber hereditary optic neuropathy (LHON) is a maternally inherited form of retinal ganglion cell degeneration leading to optic atrophy in young adults. It is caused by four primary point mutations including G11778A, G3460A, T14484C and G14459A in the mitochondrial genome (mtDNA). The mutation rate for mtDNA is about 10 times more than nuclear DNA. The D-loop region is a hot spot for mtDNA alterations and it contains two hypervariable regions (HVS-I and HVS-II). In order to identify polymorphic sites, genetic background and also to find out any possible association between LHON primary mutations and mtDNA haplogroups (hg), the complete non-coding region of mitochondrial DNA from 25 unrelated LHON patients harboring one of the primary mutations was sequenced. Any differences with cambridge sequence recorded as single base substitution (SBS), numerical changes in C-tract (PCT), insertions and deletions. Our results showed that majority of our patients belonged to hg J, T and HV rather than hgs U1, U3, U4, U5 and W, which found only in one patient (4%). Nucleotide substitutions make up the majority of the mutations. (94.5%) We have predominantly found transitions (79.2%) and a significantly lower frequency of transversions (15.3%) whereas insertions (5.5%) as well as deletions (0%) are rather rare. Ten polymorphisms were newly identified in this study not published in the mitomap database. Also PCT changes were present in all of our samples. The analysis presented here for the first time provides evidence that there is association between G3460A with hg W and U5 and also between G14459A with hg U1.

P1193. Nijmegen breakage syndrome - inherited syndrome of chromosomal instability in the population of Slovakia.

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Molecular genetic method was established for simple screening detection of slavic mutation 657del5 of NBS1 gene on both alleles. We have investigated 724 samples of dry blood blots of newborns from north Slovakia, 427 samples DNA of oncologic patients, 32 persons of 7 families within 4 probands were detected as homozygotes and 4 probands as heterozygotes, 4 of 10 siblings were heterozygote-carriers of the mutation. Immunological and oncological parameters such as IgA, IgG, IgM, IL2, IL4, IFN, TNF, CD3, CD4, CD8, CD16, CD19, CD64 and AFP, B2MG, CEA, Ferritin were investigated in patients and carriers of NBS1 gene mutation.

Incidence of the slavic mutation of NBS1 gene in the group occurred 1:181. The mutation of 657del5 was found also in gipsy ethnicum. No slavic mutation of NBS1 gene was detected in the group of oncologic patients. The most frequent defects of immunity was IgA (a)hypogammaglobulinemia ($IgA<0.08g/l$), accompanied with the reduction of CD19+ B-Lymphocytes ($BLYCD19+ <70cells/ul <10.9\%$, $TLYCD3+ <695cells/ul <67\%$, $TsLYCD8+ <330 cells/ul <29.4\%$, $ThLYCD4+ <378cells/ul <44.2\%$). No significant differences in

immunity were detected in heterozygot-carriers.

High incidence of 657del5 mutation of NBS1 gene in Slovakia correspond with other slavic countries. Detection of 657del5 mutation of NBS1 gene by used PCR method is usefull to confirm diagnosis of NBS and also for the screening to find carriers of the mutation to prevent of NBS. While homozygot-patients suffer immunodeficiencies and malignancies, heterozygot-carriers are well compensated with physiologic allele.

The research project was sponsored by Slovak Science and Technology Assistance Agency.

P1194. FSHB gene polymorphisms and haplotype patterns in European, African and Asian populations

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Follicle-stimulating hormone (follitropin, FSH) belongs to gonadotropin hormones family. Like all gonadotropins follitropin consists of α - and β -subunits. α -subunit is common in all gonadotropin hormones. β -subunit is hormone specific. Follicle-stimulating hormone β -subunit plays the important role in human sexual development and reproduction. Gene expression pattern is time and tissue specific. FSHB gene has highly conserved structure and function, and little non-synonymous variation. Nowadays all identified coding changes give rise to infertility. This circumstance is asserting follitropin β -subunit gene strong negative selection. Non-coding changes have high frequency in population and this circumstance refers either to polymorphisms evolutionary old-age or strong evolutionary selection. Despite of importance of FSHB gene in human reproduction, the general population variation and haplotypic structure of FSHB has not been investigated.

This research project aims to (a) survey FSHB variation world-wide by resequencing and RFLP analysis strategy and (b) investigate whether the identified gene variants have functional consequence. In all studied populations (Estonia, Mandenka, Han, Korea, Czech, CEPH panel) the majority of identified single nucleotide polymorphisms are frequent variants (30% minor allele frequency). Only one major haplotype (A) is present in all studied populations. However, in European populations also other major haplotype (B) was found, which coincides with chimpanzee FSHB gene sequence. The tests for selective neutrality showed that this two haplotypes (A and B) have been favoured by selective pressure. Genotype data and allelic distribution of FSHB gene have shown that haplotype B is more prevalent in women with minimum (<3 months) time to become pregnant.

P1195. The structure and the load of hereditary diseases in Seversk, Russia.

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The results of medical-genetics investigation Seversk's population are represented in this article. Seversk city are situated in region of influence of large nuclear-chemical complex - Siberian Chemical Plant. The number of investigative population was 117 thousand people. The 26 nosological forms of autosomal dominant diseases with 68 patients were revealed, and more often was the group of hereditary syndromes. Autosomal recessive pathology includes 26 nosological forms with 60 patients. The group of neurological diseases and the group of hereditary syndromes had the same number of nosological forms. X-linked diseases are represented 4 nosological forms, 10 affected persons were found. The load of autosomal dominant, autosomal recessive, X-linked pathology was 0.89; 0.89 per 1000 individuals respectively and 0.29 per 1000 men. The hereditary pathology spectrum in the populations studied was described. The total load of hereditary diseases in Seversk's population was 2.07 per 1000 individuals and was comparable with other Russian populations located in the radionuclide purity regions.

P1196. A reliable technique to identify G6PD-deficient heterozygous subjects showing the Mediterranean or non-Mediterranean phenotypes

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Differential expression due to cellular mosaicism in heterozygotes for G6PD deficiency is the basis for unreliable identification. We

compared two techniques for their identification: measurement of the enzymatic activity or that of the percentage of erythrocytes without activity (cytochimic analysis). The sample comprised 105 "obliged" heterozygous (G6PD B⁺/G6PD B⁻) having at least one normal son (G6PD B⁺; mean activity = 9.3 \pm 1.0 U/gHb; 0.0% erythrocytes without activity), and a G6PD B⁻ Med deficient son (G6PD B⁻ Med; electrophoretic mobility = 100/100; activity < 5% of 9.3 U/gHb; 99.6% \pm 1.7 of erythrocytes without activity) or G6PD B⁻ non-Med deficient son (G6PD B⁻ non-Med; electrophoretic mobility = 100/100; activity > 5% of 9.3 U/gHb; 74.1% \pm 19.6 of erythrocytes without activity). The heterozygotes were subdivided into 49 G6PD B⁻ Med, and 56 G6PD non-Med with mean activities of 5.8 \pm 2.3 U/gHb and 5.6 \pm 1.8 U/gHb, respectively, compared with 9.6 \pm 1.0 U/gHb in the normal women. The subsamples showed 51.1% \pm 33.6 of erythrocytes without activity and 31.2% \pm 27.3, respectively, compared with 0.0% of the normal women. By measurement of activity, 9/56 G6PD B⁻ Med deficient heterozygotes and 11/49 G6PD B⁻ non-Med heterozygotes, mimicking normal activity, were not identified. Test sensitivity was 80.9%. By cytochimic analysis, 2/49 G6PD B⁻ non-Med heterozygotes and 3/56 G6PD B⁻ Med heterozygotes were not identified. Test sensitivity was 95.2%. In mass screening, the cytochimic analysis is a reliable technique to identify G6PD deficient heterozygotes showing the G6PD Med or G6PD non-Med phenotype.

P1197. Finding the needle in the haystack: Selecting Ancestry Informative Markers through bulk analyses.

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In our search for Ancestry Informative Markers (AIMs), we utilised the Affymetrix Mapping 10K array, which enables the typing of more than 10,000 Single Nucleotide Polymorphisms (SNPs) per individual. Only 250 nanograms of DNA are sufficient enough to obtain the genotypes via restriction digest, anchor-primed PCR and hybridisation. In a screening of 76 cell lines, representing humans from six different geographical areas, we successfully scored over 10,000 out of 11,560 SNPs (i.e. a call rate of approximately 95%).

SNPs that were typed in less than 90% of the individuals were removed from the final dataset. Further analyses were performed on the remaining 8650 assorted SNPs. The statistical program STRUCTURE was used in order to obtain an unbiased estimate of the number of genetically distinct human sub-groups. To assess which specific SNPs could differentiate between sub-groups, F_{ST} -values were calculated via different approaches (estimators) in a biased (six sub-groups) and unbiased (four sub-groups) fashion. AIMs were identified by different F_{ST} -estimators under pre-set restrictions, which resulted in virtually no SNPs in the biased, and 47 SNPs in the unbiased classification. The 47 SNPs have a between SNP physical distance of at least 20 Mb. On the basis of these 47 SNPs STRUCTURE analysis was repeated, resulting in an identical result as obtained when using the 8650 SNP-set.

Our analyses indicate that it is not possible to identify the most optimal set of SNPs by means of a single F_{ST} estimator. However, AIMs could be successfully identified by means of a multifactorial approach.

P1198. Language and genes of the greater Himalayan region

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The greater Himalayan region includes the highest land barrier on the face of the Earth and displays a very complex socio-cultural system. It is known that populations separated by barriers (both geographical

and cultural) tend to undergo both linguistic and genetic change and, indeed, the greater Himalayan region is the area of the greatest phenotypic and linguistic diversity in the Eurasian continent.

Linguistic and archaeological studies of this region have provided strong evidence that prehistoric population movements throughout the Eurasian continent have been strongly influenced by topography. However, there are several models for the peopling of Eurasia which are still debated and, unfortunately, few genetic data are available from the greater Himalayan region.

In an attempt to shed some light on these issues, we have sampled about 1000 individuals from Nepal and 1000 from Bhutan, representing some 35 to 40 different groups (based on language, geography and/or caste). These samples are currently being analysed for genetic polymorphisms on the autosomes, the mitochondrial genome, and the Y chromosome. This will enable us to compare and test alternative models for the peopling of the Himalayan region.

Preliminary results (on 21 autosomal- and 24 Y-chromosomal STRs) support the categorisation of Himalayan populations into a Tibeto-Burman (TB) and an Indo-European-speaking (IE) family. Based on the current results, subdivision of the TB branch is more apparent in Bhutan than in Nepal.

P1199. Micro-differentiation among different sub-populations in Corsica.

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It is well known that genetic isolates have a great potential for the identification of genes involved in the pathogenesis of multifactorial diseases. In these populations the disease allele reveals Linkage Disequilibrium (LD) with markers over significant genetic intervals, therefore facilitating disease locus identification. We have already shown a high degree of LD in populations of Corsica. In the present study we have examined the amount and decay of LD with distance in some sub-populations of Corsica (Corte, Niolo, Bozio, Ajaccio) and in the general population of Corsica to verify if there are differences between these subgroups.

We have studied the Xq13.3 genomic region that has been widely used as a measure of general LD in a given population and to compare the levels of LD between populations.

We have found an extreme variability of amount of LD: Bozio and Niolo show an high degree of LD while Corte, Ajaccio and the general population of Corsica show low degree of LD. The same variability characterizes the pattern of disequilibrium: distance can explain from less than 2% up to 50% of the total LD variation in these different sub-populations. These results are likely due to a different genetic structure in these isolates: number of founders, expansion rate, amount of immigration are factors that could affect the observed micro-differentiation and LD extension.

Our results stress the importance of choosing the proper sub-populations to carry out association studies, because different demographic history of the isolated populations could affect the validity of results.

P1200. The penetrance of *HFE* C282Y mutations with respect to hepatocellular carcinoma is low in a U.K. population

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Although most patients with hereditary haemochromatosis have *HFE* C282Y mutations, the risk to *HFE* C282Y homozygotes of developing fatal diseases such as hepatocellular carcinoma is uncertain. Our aims were to determine the proportion of diagnosed hepatocellular carcinoma patients who are homozygous for the *HFE* C282Y mutation and to estimate the penetrance of this genotype with respect to hepatocellular carcinoma in East Anglia.

Tissue biopsies were analysed from 144 cases of hepatocellular carcinoma for *HFE* C282Y mutations. Data were retrieved from the East Anglian Cancer Intelligence Unit, from appropriate life tables and from our publications describing the frequency of *HFE* mutations in a large sample of the local population.

8/144 of the cases were homozygous for the *HFE* C282Y mutation, all 8 cases were male and 6 had been previously diagnosed with hereditary haemochromatosis. The frequency of this genotype was 13.1 times higher in the male hepatocellular carcinoma cohort than in the control cohort. For this cohort, we estimate that the penetrance of the *HFE* C282Y homozygous genotype, with respect to hepatocellular carcinoma, was between 1.31 % and 2.1% for males and was zero for females.

In this population, individuals who are homozygous for the *HFE* C282Y mutation have a small but significantly increased risk of being diagnosed with hepatocellular carcinoma.

P1201. Investigation for D-loop variations, haplogroup association and mitochondrial deletions in Iranian patients with Friedreich's Ataxia

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Friedreich's ataxia (FA) is one of the commonest genetic cause of ataxia and is associated with the expansion of a GAA repeat in intron 1 of the frataxin gene. Mitochondrial DNA (mtDNA) could be considered a candidate modifier factor for FA disease, since mitochondrial oxidative stress is thought to be involved in the pathogenesis of these diseases. The Displacement loop(D-loop), which is 1124 bp in size is a noncoding region contains two HyperVariable Segments(HVS-I and HVS-II). We studied 15 Iranian patients (9 females and 6 males) from 7 unrelated families. In order to identify polymorphic sites, genetic background and also to find out any possible association between FA and mtDNA haplogroups(hg), the complete non-coding region of mitochondrial DNA from 15 unrelated FA patients harboring GAA trinucleotide expansions was sequenced. Alignment were made upon the Cambridge Reference Sequence (CRS) and any differences recorded as single base substitution(SBS), insertions,deletions and numerical changes in C-tract(PCT) PCT changes were present in all of our samples. One polymorphisms (C16176A) was newly identified in this study population,not recorded in the human genome database.(Mitomap) Our results also showed that none of our patients had association with D-loop haplogroups. Deletions were present in 75% of our patients representing mtDNA damage that may be due to Iron accumulation in mitochondria and hypersensitivity to oxidative stress. The results indicate that increased levels 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 FA.

P1202. Relation between isolated orofacial clefts and maternal diseases in Hungary

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The objective of the study was to evaluate the possible association between all maternal diseases and isolated orofacial clefts (OFC) in Hungary.

The occurrence of acute and chronic maternal diseases was compared among different types of OFC, in addition between cases with OFC and population and patient controls, multiple logistic regression was used. The data set of the large population-based Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980-1996, was evaluated which includes 1,975 cases with isolated OFC (1,374 cases with cleft lip ± cleft palate (CL±CP) and 601 cases with posterior cleft palate (PCP)), 38,151 matched population controls (without defect) and 20,868 patient controls with other defects. Data collection was based on medical records, maternal data by a self-reported questionnaire and home visits in nonrespondent families.

Our study showed an increased risk for (CL±CP) in mothers with influenza, common cold, orofacial herpes and gastroenteritis and for posterior cleft palate in mothers with influenza, sinusitis and bronchitis during the critical period of these defects. Among chronic maternal diseases, epilepsy and angina pectoris showed a higher prevalence in the mothers of cases with OFC.

Some maternal diseases are risk factors for the pathogenesis of isolated OFC. It is worth considering the prevention of possible harmful effect (e.g. fever) of acute maternal diseases, such as influenza, common cold and others.

P1203. The elongation factor 2 - a candidate gene for the aging process in man?

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The aging process is characterized by an imbalance of a variety of different metabolic processes including e.g. overall protein synthesis. The EF2, catalyzing the last step of peptide chain elongation, is moreover considered to be a candidate gene of the aging process. However, up to now it is unclear whether genetic variants of EF2 might influence its biological activity. Therefore, we screened the EF2 gene for novel genomic variants (SSCP-analysis) in 470 longstanding healthy volunteers of Central Germany (mean age: 42,5±11,2 y, male: 63%). Four novel genomic variants were identified in the promoter region (PM: c.- 61C>T), in exon10 (PM: c.1632T>C, mutation: c.1677G>A) and in exon12 (mutation: c.2109T>C) by use of SSCP-analyses. The two mutations (c.1677G>A, c.2109T>C) are rare and in each case only one heterozygous carrier was identified. Genotype frequencies of both the promoter-PM (CC/CT/TT: 0,63/0,32/0,05) and the PM in exon 10 (CC/CT/TT: 0,89/0,09/0,02) are in Hardy-Weinberg-equilibrium. An evaluation of the two polymorphisms in relation to the age of the blood donors revealed significant differences in the distribution pattern only for the PM in exon 10. Homozygous carriers of TT- (38,2±12,8y) were significantly younger than CT- (41,2±10,5y) and CC-carriers (43,2±11,2y) (one-way-anova; p<0,047). Because of the higher frequency of CC-carriers at higher age, this PM could be considered to be a protective factor in the aging process. Although this PM does not result in an amino acid substitution, this genomic variant might influence the biological activity of EF2 possibly due to a change in expression on transcriptional and/or translational level.

P1204. Thymidylate synthase (TS), 2T gene polymorphism is a risk factor for acute leukemia

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Clinical and pathologic aspects of leukemia are well documented but we still need to know more about the genes that influence disease. Role of the folate pathway in leukemia susceptibility have been reported. One of the branch of folate metabolism is thymidylate synthase and it plays critical role in supplementation of deoxynucleotides required for DNA synthesis. TS gene located on chromosome 18p11.32 and it has unique tandem repeat in the 5'UTR and this area shown to be polymorphic containing either 2 or 3, 28-bp repeats and was shown to affect gene expression in vitro- in vivo studies.

We aimed to research the affect of TS polymorphism in the development of acute leukemia. Therefore we examined thymidylate synthase(TS) 28 bp repeats gene polymorphism in childhood ALL (n= 110) and in pediatric and adult AML samples(n= 126). We get DNA from each samples white blood cell and made PCR using with specific primers. Amplified PCR products were visualized on a 4% agarose gel with ethidium bromide. Results were compared with results of healthy control group (n=133). Statistical analysis were made on Fisher's exact test by SPSS for windows software.

Table 1: TS gene polymorphism in Turkish ALL, AML, control group

Genotype	ALL(n=110)(%)	AML(n=126)(%)	Control(n=133)(%)
2T/2T	22 (%20)	17 (%13,5)	22 (%16,5)
2T/3T	47 (%42,7)	72 (%57,1)	67 (%50,4)
3T/3T	41 (%37,3)	37 (%29,4)	44 (%33,1)

2T/2T polymorphism was found statistically significant for the development of ALL (p=0.048, 2-sided). On the other hand, we couldn't find any relation in between other genotypes and acute leukemia.

P1205. Investigation of Mitochondrial haplogroups among Iranian patients affected with pulmonary tuberculosis

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Introduction:

Tuberculosis is one of the most common infectious diseases in the world. Host genetic factors play a main role in the susceptibility to infectious diseases such as this disease. Also many studies have been published about relation between mitochondrial haplogroup and disorders.

Method and Material:

We studied mtDNA haplogroups M, N, J and K among Iranian patients affected with pulmonary tuberculosis. 54 patients with smear positive tuberculosis have been chosen randomly. All of patients were chosen based on having an Iranian nationality, age above 15 yrs and confirmed smear positive pulmonary TB. Also 261 healthy controls have been chosen regards to ethnicities and location of patients.

Results:

Our studies showed haplogroups M and N had similar frequencies between patients and normal control, so that 1.85% and 72.2% for haplogroup M and N, 2.44% and 83.3% for haplogroup M and N in patients and normal control, respectively.

While frequencies of haplogroups J and K showed significantly different among patients and healthy control. These results indicated 5.5% and 16.6% for haplogroups J and K in patients whilst we observed 9.39% and 7.14% for haplogroups J and K in controls respectively.

Discussion:

So far any results have been published about role of mtDNA haplogroups in susceptibility to infectious diseases such as tuberculosis, so this might be the first study to realize relation between haplogroups and susceptibility to diseases. Results show haplogroups J and K may involve in this susceptibility, but more patients should check for haplogroups J and K others.

P1206. The analysis of associations of mitochondrial DNA polymorphism with cardiovascular system function in Tuvinians

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In studying of the widely spread diseases, one of the possible approaches is to investigate genetics of normal organism functioning. Among others, energy production might be one of the key factors in defining function of cell. Cardiovascular system which is frequently involved in multifactorial diseases may respond to deviations in mechanisms of energy production. We studied population polymorphisms in mitochondrial DNA encoding for proteins of respiratory chain in association with some parameters of cardiovascular system (blood pressure measures and electrocardiography data) in ethnically pure population of Tuvinians (people from South Siberia region). 203 inhabitants of Teely village were investigated (102 males, 101 females). For mitochondrial DNA, data on HVS-I sequence (frequent polymorphic sites), T16519C polymorphism (+HaellII16517) and restriction-based haplogroups were used as possible influencing factors. One-way analysis of variance was used for detection of associations, and all variables were adjusted by age. The association was found for blood pressure measurements on both arms in males with HaellII16517 polymorphism as well as with substitution in positions 16092 or 16093 (P<0.05). There were no significant associations with ECG parameters. In addition, using abundant haplogroups as factors (haplogroups B, C, D, M) did not reveal any association either. This fact suggests that the polymorphisms in noncoding regions of mtDNA can be considered as involved into mtDNA function and, in turn, in cardiovascular system function. The study was supported by RFBR grant 04-04-48792.

P1207. Investigation of vitamin D receptor gene variants in Iranian patients affected with pulmonary tuberculosis

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Environmental and host genetic factors involve in susceptibility to infection disease such as tuberculosis. Host genetic factors including vitamin D receptor gene (VDR) shows influence in susceptibility to several disease like pulmonary tuberculosis. Interaction between vitamin D receptor and vitamin D, 1, 25 dihydroxyvitamin D3 as an important immunoregulatory hormone appear on human monocytes and activated T and B lymphocytes. Variations in the vitamin D receptor gene influence in susceptibility to infectious diseases.

It was studied to find out variants of polymorphism of this gene. *Bsm* I, *Taq* I and *Fok* I polymorphisms of VDR gene were studied in PTB patients (n= 54) and normal population who examined clinically to exclude any disease. All of patients were chosen based on having Iranian nationality, age above 15 yrs, confirmed smear positive pulmonary TB and all of healthy controls were matched in nationality and age with samples. DNA was extracted from whole blood and PCR- RFLP technique used to determine variants of mentioned polymorphisms of VDR gene.

Our studies showed a significantly difference in two *Taq* I and *Bsm* I polymorphisms between patients and healthy controls, so that the frequencies for TT, tt and BB, bb were 53.3%, 7.6% and 50%, 7.6% in patients, while these frequencies were 12.9%, 31.4% and 22.2%, 33.3% in controls, respectively. *Fok* I polymorphism hasn't showed any different among samples and controls. Our results showed that homozygosity in two *Taq* I and *Bsm* I polymorphisms can play a main role in susceptibility to tuberculosis in our patients.

P1208. Genetic diversity and differentiation of Y-chromosomal lineages in North Eurasia

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Composition and frequency of Y-chromosomal haplogroups, defined by the genotyping of 36 biallelic loci in non-recombining part of Y-chromosome, was revealed for native population of Siberia, Central Asia and Eastern Europe. Slavonic ethnic groups, which geographically represent Eastern Europe, are characterized by the high frequency of R1a1, I*, I1b, and N3a clades and by the presence of R1b3, J2, E, and G. Most frequent haplogroup is R1a1, which comprises 44-51% of Y-chromosomes. The distinguishing peculiarity of Central Asian Caucasoids is the high frequency of Caucasoid clades R1a1, J*, J2, and the presence of R1b3 and G. Twenty-five haplogroups were found in gene pool of native Siberian populations. Only 7 of them have the frequency higher than 3%. In sum these 7 clades comprise 86% of Siberian samples. In populations of Southern Siberia the most frequent haplogroup is R1a1. The high frequency of N3a is characteristic for Eastern Siberians, and in Yakuts its frequency is almost 90%. Koryaks, Buryats and Nivkhs have the highest frequency of C3* lineage among investigated populations. Haplogroup O* revealed with variable frequency in most of Siberian. Highest frequency of Q* was found in Kets and Northern Altayans (85% and 32%, respectively). The high level of genetic differentiation of North Eurasian population on Y-chromosomal lineages was revealed. The proportion of inter-population differences in the total genetic variability of region's population according to the analysis of molecular variance is 19.04%. Genetic differences between territorial groups took 6.9% of total genetic variability, whereas 12.8% is the inter-population differences within groups.

P1209. The involvement of estrogen receptor 1 (ESR1) in Alzheimer's disease is mediated by apolipoprotein E (APOE).

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In the present investigation we studied the role of ESR1 in the susceptibility to sporadic Alzheimer's disease (AD), its interactions with APOE gene polymorphism, and its effect on APOE plasma levels. Genotype and allele frequencies of ESR1 polymorphisms Pvull and XbaI did not differ between AD patients (n=279) and controls (n=212). The interactions between ESR1 and APOE polymorphisms in AD susceptibility were evaluated by logistic regression analysis which

showed that the presence of ESR1 PP and/or XX genotypes together with APOE e*4 allele confers an elevated risk for AD (O.R. = 12.8, 95%CI 1.7-98.0, p=0.01) three times higher than that associated with the presence of e*4 only (O.R.= 3.9, 95%CI 2.3-6.5, p<0.00001). In both AD patients and controls APOE plasma levels were determined as well. AD patients carrying e*4 showed significantly lower APOE concentrations (3.0 ± 1.2 mg/dl,) compared with non-e*4 carriers (4.1 ± 2.0 mg/dl). Considering ESR1 genotypes, PP and XX homozygotes showed significantly reduced APOE concentrations (3.0 ± 1.4 and 3.1± 1.5 mg/dl respectively). Patients carrying both APOE e*4 allele and ESR1 PP or XX genotypes showed the lowest APOE concentrations (2.7± 1.2 and 2.8 ± 1.32 mg/dl) suggesting an additive effect of the two genes on APOE levels. Since in brain APOE has a neuroprotective action, we suggest that the increased risk for AD associated to the presence of ESR1 PP and/or XX genotypes together with APOE e*4 allele could be explained by the remarkable reduction of APOE levels associated to them.

P1210. The frequency of CCR5 -Δ32 in Western Anatolia

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It has been shown that a 32 base pair deletion in the C-C Chemokine Receptor 5 (CCR5) gene plays an important role in determining susceptibility to infection by human immune deficiency virus type I (HIV-1). In this study, we aimed to assess the frequency of the CCR5-Δ32 allele in healthy Turkish population living in Western Part of Turkey. The mutation was investigated in 515 noninfected unrelated (ages between 13-40, male to female ratio 1.1/1) individuals from related area.

The frequency of the CCR5-Δ32 in study group was found to be 6.4%. This frequency was detected to be around ≈10% in European Countries whereas it was 0-4% in Southern Asia and Africa. The frequency found in Turkish population is between the frequencies described in Northern Countries and Southern Countries and supports the historical and geographical distribution of Δ32 allele of CCR5 proposed by different authors.

This is the first study investigating Δ32 allelic frequency in Western Part of Anatolia and shows a correlation with the study covering Ankara (Middle Anatolian) population. Further studies covering the other parts of Anatolia will contribute to demonstrate the regional differences or correlations in Turkey.

P1211. Markedly high frequency of SMNt deletion in the Iranian population

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Spinal muscular atrophy (SMA) is a hereditary neurodegenerative disease often causing death in early childhood. This disease is caused by anterior -horn-cell death in the spinal cord leading to paralysis and skeletal muscular atrophy. The candidate gene for this disease is survival motor neuron gene (SMN) that exists in two nearly identical copies; telomeric SMN (SMNt) and centromeric SMN (SMNc). Because of gene deletion or conversion, exon 7 of SMNt gene is homozygously absent in approximately 94% of typical SMA patients. SMA affects approximately 1 in 10,000 live births with a carrier frequency of approximately 1 in 50. In this study we decided to determine the carrier frequency of this disease which is extremely common in Iran. Two hundred unrelated individuals were selected from different ethnic populations of Iran. In all individuals, exon 7 of SMN gene was amplified by polymerase chain reaction (PCR) and PCR products were digested by Dral restriction enzyme and then electrophoresed on 8% polyacrylamide gel. In all steps, control samples which were normal for SMN alleles, heterozygote and homozygote for the deletion of exon 7 were used. To differentiate carriers and normal individuals, the intensity of digested fragments was analyzed by LabWorks software to calculate the ratio of telomeric to centromeric portion of SMN gene.

Surprisingly, the carrier frequency of this disease seems to be around 12%, and not evenly distributed among the ethnic subgroups that is much higher than the carrier frequency of 1/40 to 1/60 that has been reported before in the West.

P1212. Are the recommendations on the prevention of neural tube defects working?

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Many studies showed that reduction by an estimated 80% or more of neural tube defects (NTD) by consumption of folic acid from before conception is achievable.

The objectives of this study were to evaluate the effectiveness of recommendations on folic acid aimed at reducing the occurrence of NTD in our region.

Cases of NTD were ascertained among liveborn infants, stillbirths, and terminations of pregnancy. Incidences and trends in rates of NTD before and after 1992 (the year of the first recommendations) and before and after 1995 (the year of local recommendations) were obtained. The results showed that the issuing of recommendations on folic acid was followed by no detectable improvement in the trends of incidence of NTD. The rates of NTD per 10,000 were before 1992 9.7, from 1993 to 1995 10.1, and after 1995 13.6, respectively.

In conclusions new cases preventable by folic acid continue to accumulate. Recommendations alone did not influence trends in NTD in our region up to nine years after the confirmation of the effectiveness of folic acid in clinical trials. New strategies are needed.

P1213. Azorean ancestry assessed by Alu insertion polymorphisms

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Alu insertion polymorphisms have been extensively used to unravel the origins of populations, since they reflect both maternal and paternal history of a population. The Azores, an Atlantic archipelago, had no native population and were mainly populated by the Portuguese people. African slaves, Moorish prisoners, French and Spanish also contributed to the initial settlement during the 15th century. In order to characterize the population admixture we typed 7 Alu (TPA-25, ACE, APO, B65, PV92, FXIIIB and D1) in 95 unrelated Azorean blood donors. Allele frequencies were calculated by direct counting. Statistical analysis was performed using Arlequin v.2.0, and Nei's genetic distance trees were constructed with DISPAN software by Neighbor algorithm.

Hererozogosity values ranged from 0.084 for APO to 0.484 for ACE and all markers were in Hardy-Weinberg equilibrium. Average gene diversity varied from 0.1196 for APO to 0.4985 for B65. Considering all loci, gene diversity was 0.9658 indicating high variability in the Azorean population. In addition, allele frequencies are similar to those obtained for European and North African populations, except for FXIIIB which shows a much higher value (0.774). These results are corroborated by the dendrogram where Azores branches with Catalans, Andalusians, Moroccans and Algerians. Interestingly, Azores is located apart from other European populations, such as French and Germans. Moreover, the distinctive position of the archipelago also suggests that, despite the high similarity with European and North African populations explained by the historical data of settlement, the Azores presents a unique genetic signature. (claudiacbranco@hdes.pt). Funded by DRCT (Azores).

P1214. Analysis of CARD15 Mutations in Italian Inflammatory Bowel Disease (IBD) patients

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Crohn's disease [CD (MIM #266600)] and Ulcerative Colitis [UC (MIM #191390)] are the two major forms of chronic inflammatory bowel disease (IBD) with a combined general prevalence of approximately 100-200 of 100,000 people in Western countries. The

aetiopathogenesis of CD and UC is largely unknown. Several lines of evidence have suggested they are multifactorial diseases in which both genetic and environmental factors appear to play a role in disease development. *CARD15* gene (MIM *605956) has been identified as the first determinant of susceptibility for CD in populations of European descent and in particular, three mutations (Arg702Trp, Gly908Arg and Leu1007fsinsC) have been strongly associated with CD younger age at onset, ileal location and a fibrostenotic or fistulizing disease behaviour. We therefore investigated the presence of Arg702Trp, Gly908Arg and Leu1007fsinsC in an Italian IBD population (166 and 92 patients with CD and UC respectively) Allele frequencies were compared to those observed in a control population (164 individuals). Gly908Arg and Leu1007fsinsC were significantly more frequent in CD patients compared to healthy controls ($p<0.05$ and $p<0.001$ respectively). Estimated risks (OR) for the two mutations were calculated by a logistic regression analysis (IC 95%) and data demonstrated an increased risk of developing CD in Gly908Arg and Leu1007fsinsC carriers [OR= 4.78 (CI 1.15-19.8); OR= 7.79 (CI 1.96-31.0) respectively]. As far as concerns genotype-phenotype correlation we found association with ileal location and a fibrostenotic or fistulizing behaviour. We finally confirm the significant association of *CARD15* gene mutations and CD.

P1215. Lack of association between the polymorphisms Glu298Asp and 4VNTR of ENOS gene and premature coronary artery disease in the Greek population

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Genetic polymorphisms in the gene for endothelial nitric oxide synthase (ENOS) have been considered as a potential risk factors for the development of coronary artery disease (CAD) in some populations. We studied two polymorphisms of the ENOS gene, the Glu298Asp and the 4VNTR, in a total number of 269 individuals of the Greek population. The patient group was consistent of 187 subjects, aged under 58 years presenting with symptomatic CAD, documented by coronary angiography. The frequencies for GG (Glu/Glu), GT(Glu/Asp), TT(Asp/Asp) of the Glu298Asp polymorphism were 0.56, 0.38, 0.06, respectively, for the patient group and 0.49, 0.46, 0.04, for the control group. The frequencies for bb, ab and aa genotypes of 4VNTR polymorphism were 0.66, 0.29, 0.04, respectively, in patients compared to 0.73, 0.24, 0.03 in controls. The statistical analysis of these results show that there are no significant differences in the frequencies of the genotypes between patients and controls, for these two polymorphisms of ENOS gene. Thus, in contrast to some earlier findings by others from other populations, we have found no evidence for an association between the a allele of the 4VNTR polymorphism, or between the T allele of Glu298Asp polymorphism and the risk of premature coronary artery disease, in the Greek population.

P1216. Genescape of India, as Reconstructed from Polymorphic DNA Variation in the Y chromosome

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The contemporary male gene pool of ethnic India largely comprises haplogroups that originated indigenously, in southeast Asia, and in west and central Asia. The indigenous haplogroup is predominant among the tribal group. The southeast Asian influence is largely on the male gene pools of Tibeto-Burman speaking tribals and Austro-Asiatic and Dravidian. The west and central Asian influence is primarily on caste groups - both Indo-European and Dravidian.

The haplogroup diversity within the various tribal groups is lower than that within the caste groups. Analyses of molecular variance showed higher genetic variability among populations within linguistic clusters of tribals compared to castes. Moreover, the between group variability in the Indo-European caste cluster is higher than that in the Dravidian caste cluster. This may be a reflection of diverse ancestries, antiquities and isolation of the tribals, coupled with subsequent cultural (linguistic) homogenization. Lesser between group genetic variability in caste

groups may be a reflection of their recent founding history.

The complete congruence of the patterns of Y-chromosomal and mitochondrial DNA differentiation may be indicative of inflow of both male and female genes from similar source populations. The rank order of FST values showed that tribes and castes are most differentiated, followed by upper and middle caste, upper and lower caste and middle and lower caste.

P1217. Genetic polymorphism of xenobiotics metabolism enzymes and susceptibility to COPD and lung cancer

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Objective: Cigarette smoking is the major risk factor for the development of both chronic obstructive pulmonary disease (COPD) and bronchogenic carcinoma, but only 10-20% of heavy smokers develop the diseases, which suggests the presence of genetic susceptibility. The genetic susceptibility to lung cancer and COPD might depend on variation in antioxidative enzyme activities that detoxify cigarette smoke products such as microsomal epoxide hydrolase (mEH) and glutathione S-transferase M1 (GSTM1).

Methods: The polymorphisms in GSTM1 and mEH genes were examined in 156 lung cancer patients, 148 patients with COPD and 150 healthy control subjects using PCR and PCR-RFLP methods. The genotype frequencies of mEH and GSTM1 genes were compared between patients and controls.

Results: We suggest that the presence of at least one mEH „slow allele“ significantly increases lung cancer risk (OR=1.71), especially among smokers (OR=1.83). Moreover, carriers of both slow alleles with very low mEH activity have increased lung cancer risk (OR=2.14), but significantly only among nonsmokers. The odds ratio of individuals with both potentially risk genotypes: homozygotes for slow allele in mEH gene and for null allele in GSTM1 gene *versus* that of other genotypes combined was 3.53. The risk of development of COPD in lung cancer patients was associated only with GSTM1 0/0 genotype (OR=2.98).

Conclusions: Lung cancer risk is associated with the presence of slow mEH allele and GSTM1 0/0 genotype and that in patients with lung cancer the presence of at least one active allele in GSTM1 has a protective effect against the development of COPD.

P1218. DNA degradation studies on human tooth samples from different periods of time.

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We propose in our study to investigate time factor influence on DNA degradation of several human tooth samples from different periods of time - Neolithic period, Bronze Age, First Iron Age, Romano-Byzantine period, the 8th-10th century and 21th century. The samples, with the exception of the most recent ones, were obtained from several archaeological sites belonging to the same geographic area (South-East of Romania). The DNA degradation degree of all of them was estimated quantitative by spectrophotometric readings (informative reasons only) and qualitative by quantification of PCR amplification ratio of mitochondrial and nuclear DNA. We use as mitochondrial DNA markers HVR I and HVR II regions (which were split in two fragments each) and TH01, D1S1656, D6S366, FES/FPS, Amelogenin, as nuclear DNA markers. The start hypothesis was that the number of DNA markers amplified by PCR will decrease with the oldness of samples. Our results confirmed this assumption - the oldest samples are, the less positive amplifications were obtain, involving amplification especially of mitochondrial fragments and in case of nuclear fragments the smallest ones.

We consider this type of knowledge being of great interest because in human paleogenetics, investigations about particularities and processes involved in bone DNA degradation in time, comparatively, for example, with that about famous historical cases, are just a few, insufficiently for problems rise by this matter.

P1219. Maternal and paternal lineages in ancient and modern Hungarians

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Hungarian language represents the westernmost group of the Finno-Ugric language phylum, surrounded entirely by Indo-European-speaking populations. Their linguistic isolation in the Carpathian basin suggests the possibility that they might also show a significant genetic isolation.

According to historical data at the end of the 9th century Hungarian conquerors from the west side of the Ural Mountains settled down into the Carpathian Basin and took the hegemony. To determine the genetic background of Hungarians we examined mitochondrial and Y chromosomal DNA from ancient 'conquerors' from Hungary, originated from the 10th century and from modern Hungarian-speaking adults from today's Hungary and Transylvanian Seklers (Romania).

DNA was extracted from 35 excavated ancient bones and hair samples of 125 and 80 modern Hungarians and Seklers, respectively. Mitochondrial haplogroups were determined with HVS I sequencing and RFLP typing.

The mtDNA HVS I sequences were compared with 2615 samples from 34 Eurasian populations retrieved from published data. ARLEQUIN 2.001 Software was used to estimate genetic distances between populations. The resulting matrix was summarized in two dimensions by use of Multidimensional Scaling.

The M46 biallelic Y chromosomal marker (TAT, often called Uralic migration marker) was also investigated from 2 ancient, 34 modern Hungarian and 60 Sekler samples. Our results suggest that the modern Hungarian gene pool is very similar to other central European ones concerning the mitochondrial and Y chromosomal markers, while the ancient population contains more Asian type elements.

P1220. HFE mutations in the São Miguel's population: genetic frequencies and geographic distribution.

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Hereditary hemochromatosis (HH, OMIM 235200) is an autosomal recessive disorder characterized by increased iron absorption. Three main mutations in the *HFE* gene (6p21.3) are implicated in HH: C282Y, H63D and S65C. Here, we estimated the frequencies and geographic distribution of these mutations in São Miguel's population (131,609 inhabitants, 2001 Census). In total, 469 blood donors from the six municipalities of the island were analysed by PCR/RFLP method. The allele frequency was 5.01% for C282Y, 22.28% for H63D and 3.20% for S65C. We found twelve genotypes: wild/wild (49.89%), wild/H63D (29.64%), wild/C282Y (7.68%), H63D/H63D (5.54%), wild/S65C (2.35%), H63D/S65C (1.71%), C282Y/H63D (1.49%), S65C/S65C (0.64%), C282Y/S65C (0.43%), and C282Y/C282Y, H63D-S65C/S65D and H63D-S65C/H63D all three with a frequency of 0.21%. The six municipalities of the island show different values for the C282Y, the most severe mutation. To study the significance of these C282Y frequency values, we carried out a statistical analysis (Fisher's exact test). We observed a statistical difference ($p=0.048$) between Nordeste (9.8%) and the other five municipalities (4.6%). This result may be explained by the relative geographical isolation of Nordeste in the island, and by its reduced population (4% of total population). Considering São Miguel's settlement history, we are genotyping the HLA-A and HLA-B loci in order to characterize the haplotypes associated with *HFE* mutations in the island. (lmotavieira@hdes.pt). Funded by DRCT (Azores).

P1221. Genotypes and alleles distribution of the KCNE1 (minK) gene polymorphism in St.Petersburg children population.

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The protein product of the minK gene is a modulator of the potassium channel activity and plays an important role in cardiac electrophysiology.

Genetic polymorphism of the minK gene is associated with clinical atrial fibrillation (Lai et al., 2002). We have previously reported the correlation between the minK 38G allele and the chronic atrial fibrillation in the patients with sick sinus syndrome (Burova et al., 2004).

The aim of this study was to determine genotypes and alleles distribution in St. Petersburg children population and compare at that of Chinese adult population.

126 healthy children (62 boys and 64 girls) aged 5 to 16 were included in our study. The minK genotype was analyzed using PCR amplification and digestion with endonuclease MspA1I. Allele frequencies and genotype distribution were compared by using χ^2 test.

We found no significant differences in genotypes and alleles frequency distribution between St.Petersburg population and Chinese population.

Our data may be used as control in investigation of minK gene polymorphism in patient with different clinical forms of heart rhythm disorders.

Alleles	St.Petersburg children population(n=126)	Chinese adult population (n=108) (Lai et al., 2002)	P-value
38G allele (%)	162 (64,3)	136 (63,0)	0,841
38S allele(%)	90 (35,7)	80 (37,0)	
Genotypes			
38G/38G (%)	53 (42,1)	46 (42,6)	
38G/38S (%)	56 (44,4)	44 (40,7)	0,747
38S/38S (%)	17 (13,5)	18 (16,7)	

P1222. Y chromosomal variation in the Czech Republic

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In order to analyse the contribution of the Czech Republic to the genetic landscape of Europe, we typed 257 male subjects from 5 locations for 17 Unique Event Polymorphisms of the Y chromosome. Sixteen haplogroups or sub-haplogroups were identified, with only 5 chromosomes uncharacterized. Overall, the degree of population structuring was low. The three commonest haplogroups were R1a (0.344), P*(xR1a) (0.281) and I (0.184). M157, M56 and M87 showed no variation within haplogroup R1a. Haplogroup I was mostly represented as I1b* and I1b2 was also detected in this population. Thus, the majority of the Czech male gene pool is accounted for by the three main haplogroups found in western and central Europe, the Balkans and the Carpathians. Haplogroup J was found at low frequency, in agreement with a low gene flow with the Mediterranean.

In order to draw inferences on the dynamics of the Czech population, we typed 141 carriers of the 3 most common haplogroups for 10 microsatellites, and applied coalescent analyses.

While the age of the I clade agreed with that reported in the vast study of Roots et al (2004), the ages of its sub-haplogroups differed considerably, showing that the I chromosomes sampled in the Czech Republic are a subset of those found throughout Europe. Haplogroup R1a turned out to be the youngest with an estimated age well after the Last Glacial Maximum. For all three major haplogroups the results indicate a fast population growth, beginning at approximately 60-80 generations ago.

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P1223. Allelic polymorphisms of metabolizing enzymes in a Hungarian roma population

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The roma population is the largest ethnic minority in Hungary. Their mortality structure differs from the national averages. In the present study we investigated the allelic polymorphisms of N-acetyltransferase 2 (NAT2), a glutathione-S-transferase M1 (GSTM1), glutathione-S-transferase T1 (GSTT1) and cytochrome P450 1A1 (CYP 1A1) metabolizing enzymes in the largest group of the Hungarian romas. The allelic frequencies were compared to the data of non roma population. Sample collection was performed in the framework of Roma Project (195 blood samples), genotyping was made from peripheral leukocytes, with PCR-based methods ((NAT2: rapid and slow acetylators, GST M1 and T1: 0 and + genotypes, CYP 1A1: Ile/Val polymorphism).

Our results demonstrated a difference between the allelic distributions of roma and non roma populations. Allelic frequencies of GSTM1 (OR: 2.08, 95% CI: 1.45-2.99) and NAT2 (OR: 1.42, 95% CI: 1.00-2.00) were significantly different, while in case of GSTT1 and CYP 1A1 there was no statistically significant difference. Since both GSTM1 and NAT polymorphisms might have an influence on susceptibility to certain cancers (e.g. colorectal, bladder and lung cancer), the found differences in the allelic distributions may contribute to the mortality differences between the Hungarian romas and non roma populations. This hypothesis, however, must be confirmed, e.g. by studying allelic polymorphisms among cancer patients from the roma population.

P1224. Prevalence of Myotonic Dystrophy type 1 in Slovenia

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The discovery of the Myotonic Dystrophy type 1 (DM1) gene and the identification of the mechanism of mutation enabled more accurate estimation of the epidemiological rates. Worldwide, the prevalence rates range from 2.2-5.5/100 000 inhabitants in Western Europe to 189/100 000 inhabitants in the Saguenay-Lac-Saint-Jean region in Quebec, Canada. In the South-East of Europe, in Istria, Croatia, one of the highest prevalence rates of 18.1/100 000 has been reported. The purpose of this study was to calculate the DM1 prevalence rate in Slovenia. Patients were ascertained over a 10-year period (1994-2004) from different sources. Direct mutation analysis was the method of choice for the diagnostic testing of DM1. A total of 91 DM1 patients from 46 families were analysed. After the correction for underascertainment, the prevalence of 8.25/100 000 inhabitants was found. The prevalence rate of DM1 in Slovenia is higher than the estimated average rate in Western Europe and comparable to the prevalence rates in the North-East and Central Italy (9.31/100 000). The improved diagnostic methods provide both accurate estimation of epidemiological rates and identification of mildly or congenitally affected patients.

P1225. BARCODE: a new software for comparative dating of haplotypic nested lineages

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We present a new software for comparative dating estimates of haplotypic lineages defined by Unique Event Polymorphisms (UEP), on the basis of linked STR diversity. BARCODE (Basic ASD Regression for Comparative Dating Estimates), applies a comparative dating approach free of assumptions on STR mutation rates to detect the linear accumulation of ASD over time. Its power relies on the exploitation of the UEP-defined phylogeny, which provides a unique solution for the order of nodes in the tree to be analysed. The set of possible solutions to be tested for ASD-TIME linearity is created through an algorithm specifically created. The more the phylogeny has a nested shape, the more the dating result is robust. BARCODE allows to weight each lineage according to its frequency in the dataset, a fundamental option

when the sampled lineages include variants supposed to be ancestral to a demographic expansion or when dealing with data unbalanced in sample-size.

We report the results obtained by running BARCODE on published Y-chromosomal data sets. The relative dating estimates are in general agreement with the absolute estimates originally published. The results also allow inferences on STR mutation rates (ASD-TIME regression slopes): some critical loci emerged, which show variable mutation patterns across the data sets. However, most STR loci show a similar behaviour across the data sets, thus confirming their informativeness. The most commonly used STR loci can then be ranked in different classes, according to their mutation rates, independently on the particular data set considered.

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P1226. A study of multiple polymorphisms of folate-dependent, one-carbon metabolism in a northern European population.

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Folates play a key role in disease prevention; deficiency is associated with increased risk of neural tube defects, vascular disease, cancer and anaemia. Many enzymes of folate-dependent, one-carbon metabolism are functionally polymorphic. Low activity forms of *MTHFR* have been associated with raised homocysteine and vascular disease. Folate metabolism is also a therapeutic target for drug groups that are widely used in the treatment of cancer and other chronic diseases. Polymorphisms leading to over-expression of *TYMS* have been associated with resistance to the chemotherapeutic agent 5FU.

The study of multiple polymorphisms simultaneously will allow us to consider additive, synergistic and compensating variants of folate metabolism that may be important clinically. Therefore, we have developed allelic discrimination and gel analysis assays for functionally polymorphic forms of *MTHFR*, *TYMS*, *DHFR*, *MTHFD*, *SLC19A1* (*REFC*), *MTR*, and *SHMT1*; most assays are for non-synonymous SNPs. The allele frequencies have been determined for 459 men (mean age 60 years; range 40 to 80 years) from Norfolk, England.

Results; loci and allele frequencies

GENE/ SNP I.D.	Rare allelic form	Frequency of rare allele
<i>MTHFR</i> /rs1801133	C677T	0.33
<i>MTHFR</i> /rs1801131	A1298C	0.32
<i>TYMS</i> /promoter 2R/3R	2R	0.46
<i>DHFR</i> /intron 1 ins/del	del	0.43
<i>SLC19A1</i> /rs1051266	G80A	0.46
<i>MTR</i> /rs1805087	A2756G	0.18
<i>MTHFD1</i> /rs2236225	A1958G	0.42
<i>SHMT1</i> /rs1979277	C1420T	0.32

Conclusions

- The robust and economical methods for studying these polymorphisms of one-carbon metabolism are presented.
- The allele frequency data and the methods can be used in disease association and pharmacogenetic studies of northern Europeans.

P1227. Screening for point mutations in the LDL receptor gene in Bulgarians with severe hypercholesterolemia

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Familial hypercholesterolemia (FH) is an autosomal dominant disease caused by mutations in the low density lipoprotein receptor (LDLR) gene. As a result, a defect in the uptake of the low density lipoprotein (LDL) and a disturbance in the lipid metabolism are present. More than 1000 mutations have been described so far (<http://www.umd.necker.fr>). There is a genetic variability between different populations

of FH patients worldwide. It is not known much about the distribution of mutations in Bulgarian population. The purpose of this study was to define the spectrum of mutations among Bulgarians. We analyzed a group of 97 people all clinically diagnosed as FH patients. The screening of mutations was achieved by a Single Strand Conformation Polymorphism (SSCP) and samples with abnormal patterns were sequenced. In 12 cases the diagnosis FH was genetically confirmed. Our results present that several exons occurred to be mutation hot-spots (exons 5, 9 and 11) in Bulgarian patients with FH. We found 6 point mutations in 6 out of 14 screened exons: ex9 1195G>A in 5 patients; ex11 1646G>A in 3 patients; ex4b 590G>A, ex6 858C>A, ex8 1073G>A, ex5 761A>C and intron 7 1061-8 T>C in single cases. The mutation 1073G>A is not described in literature. In addition two polymorphisms were found: ex13 1959C>T and ex11 1646G>A. The last one, in single case, was combined with mutation ex11 1646G>A.

P1228. Thrombophilia markers in pediatric ischemic stroke patients

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Ischemic stroke in childhood is less common than in adults (estimates of the incidence is 0.6-1.2/100,000 per year), but is a very important cause of lifelong morbidity. It is multifactorial disease in which both acquired and genetic influences may play important roles. The role hemostasis plays in pathogenesis of stroke (especially in case of thrombophilia) is still controversial.

Aim of our study was to establish the prevalence of genetic risk factors (FV Leiden, FII G20210A, *MTHFR* C677T mutations) and frequent polymorphisms (PAI-1 4g/5g and ACE ID) associated with thrombophilia in a group of children with ischemic stroke.

A study was carried out in a group of 30 children (11 girls and 19 boys, median age of first stroke episode 7.5 years) and 120 consecutive healthy blood donors.

The results are shown in Table:

		cases, %	controls, %	OR,(95% CIs)
FV Leiden	m/n	3.3	5.8	0.56,(0.08-4.71)
	m/m	0	0	-
FII G20210A	m/n	6.7	4.2	1.64,(0.30-8.91)
	m/m	0	0	-
<i>MTHFR</i> C677T	m/n	60.0 ¹	39.2 ¹	2.33,(1.03-5.28)
	m/m	6.7	11.7	0.54,(0.12-2.53)
PAI-1 4g/5g	5g/5g	23.3	18.3	1.42,(0.53-3.75)
	4g/5g	56.7	43.3	1.71,(0.76-3.85)
	4g/4g	20.0	38.3	0.39,(0.15-1.04)
ACE ID	II	23.3	25.0	0.91,(0.35-2.37)
	ID	50.0	56.7	0.76,(0.53-1.72)
	DD	26.7	18.3	1.64,(0.63-4.21)

m-mutated allele, n-normal allele, l-insertion, D-deletion, ¹p=0.039

Heterozygous carriers of *MTHFR* C677T mutation were overrepresented in children with ischemic stroke (60% patients, 39.2% controls); while difference in frequencies of other tested genetic markers was not statistically significant.

Our study suggests that thrombophilia may not play an important role in the pathogenesis of stroke in children.

P1229. HLA and surnames are compared as paradigms of the genetic history of Lombardy (Northern Italy)

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Background Lombardy was a crossroad of ancient populations that occupied the Alps and the Po valley: Ligures, Celts, Etrurians and Romans. Aim of the present work is to study its genetic structure through two kinds of markers: surnames and HLA genes, assuming that the presence of population clusters is related to the history.

Methods. 260,732 telephone subscribers constitute the surname sample: the frequencies of 10431 different surnames are calculated

in each of the 11 provinces. The HLA sample is constituted by 33,488 bone marrow donors born and resident in Lombardy and serologically typed for HLA-A and HLA-B loci (46 alleles). For both markers the correlations between provinces are represented by a principal coordinate (PCA) plot and the results obtained with HLA and surnames were compared using the Mantel test.

Results Both the surname and HLA PCA showed 3 clusters: a) the southern provinces mainly flat; b) the northeastern provinces (partly occupied by the Alps) and c) the area around the Como lake also in the Northern part. The Mantel test showed that the results obtained with HLA and surnames were highly correlated ($P=0.02$).

Discussion The 3 clusters, more evident with HLA genes, can be related to the ancient history, with a Celtic influence in the NE and Ligures-Etrurian feature in the south. The Como lake area was of Celt-Ligures origin, as demonstrated also by the dialect. The knowledge of homogeneous geographic clusters in terms of HLA gene/haplotype distribution could be useful in donor recruitment and in transplantation policy.

P1230. Molecular dissection of the Y chromosome haplogroups A, E and R1b

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The male-specific region of the human Y chromosome (MSY) is characterized by a low amount of sequence diversity compared to the mtDNA, the autosomes and the X chromosome. Recently, the use of DHPLC and direct sequencing of DNA has permitted to identify more than 300 new single nucleotide polymorphisms (SNPs) on the MSY. The analysis of the geographic distribution of the haplogroups identified by these markers has provided new insights in the history of human populations, at the same time, it came out that undetected Y chromosome SNPs still contain useful information.

In this study we have analyzed the sequence variation of 60 kb of the TBL1Y gene. While previous studies have analyzed the sequence variation of the Y chromosome in a random sample of individuals, we here focus on 22 chromosomes belonging to three specific haplogroups (A, R1b and E), whose geographic distribution is relevant for the human evolutionary history of Africa and/or western Eurasia.

We discovered 32 new SNPs, and placed them in the known Y chromosome phylogenetic tree: about half of the new mutations identify new branches of the tree. The geographic distribution of five new E-M78 sub-haplogroups, analyzed in more than 6,000 subjects from Eurasia and Africa, has led to the identification of interesting evolutionary patterns.

P1231. Universal screening programm for early detection of congenital deafness in Flanders: contribution of genetic causes

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Serious congenital hearing problems occur in about 1/1000 newborns. In Flanders (Belgium) about 65 children each year are born with congenital deafness.

Early detection of the hearing loss and its cause is important. On one hand because early intervention by proper hearing aids is crucial for an optimal social, emotional, motor- and mental development; on the other hand knowing the cause of the deafness can provide information about prognosis, recurrence risk, and possibilities of prenatal diagnosis and about possible additional problems in the future.

Since 1999 in Flanders a universal screening programm is running for newborns at the age of 4 weeks. The test consists of an AABR (automatic Auditory Brainstem Response) test, also called the Algo-test.

After 2 'refers' the patient is referred to one of the specialised centers in Flanders, where the hearing loss will be confirmed, and additional diagnostic examinations are performed by a standardized protocol, in which a geneticist plays an important role.

Until now more than 100 children with congenital deafness were detected by the Algo-test, and the results of the additional tests will be presented, including the percentage of genetic causes that were found.

P1232. Y-microsatellite polymorphism in Russian populations from northern and southern regions of European part of Russia

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An information about Y-chromosome microsatellite variation in the Russians is crucial for reconstruction of population history of Eastern Slavs. Allele polymorphism and haplotypes of five Y-microsatellites (DYS 393, DYS 392, DYS 391, DYS 390 and DYS 19) were analyzed in two Russian male populations from northern (Archangelsk) and southern (Kursk) regions of Russia. Significant differences in DYS 393 and DYS 392 allele distribution were revealed between populations tested ($p = 0.003$ and 0.005, respectively). The allele diversity indices for these loci were more than 1.5 times higher in Archangelsk population than in Kursk population. When evaluating genetic relationships between populations studied and some European populations, including already investigated Eastern Slavonic samples, it was shown that Archangelsk population was nearer to Finno-Ugrians (the Saami and Estonians) and the Lithuanians, whereas Kursk population was clustered together with Eastern Slavonic populations (the Russians from Novgorod region, the Ukrainians and Byelorussians), regardless of used genetic distance measure. The phylogenetic analysis of the most frequent haplotypes allowed us to demonstrate that differences between populations from Kursk and Archangelsk regions were due to high frequency of major haplotypes, typical for Finno-Ugric populations, in the Archangelsk population. It is suggested, that differences revealed can be caused by geographical specificity of demographic processes.

P1233. Familial aggregation of alopecia areata

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Alopecia areata (AA) is a common dermatological disorder. The etiology remains unsolved, but there is a strong evidence indicating that it is a tissue specific autoimmune disease with a genetic predisposition. Clinically it presents as a sudden onset of patchy, non-scarring hair loss, which may be diffuse or total.

Familial aggregation has been described in previous reports, supporting the genetic influence, with the rate of positive family history varying between 3-42%. Here, we report the first family study of AA to examine first-degree relatives by direct interview and to obtain information on affection status of second-degree relatives by interview of first-degree relatives. Since a key issue is proper control for age, we used survival analysis methods that allow for age-specific risk calculations. Thereby we present age adjusted risks for first and for second-degree relatives that can be used for risk counseling in families with AA.

P1234. Epidemiology of CF gene in Romania

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Paper aim: updating information about the epidemiology of CF gene in Romania. Study group: 45 CF patients (20 males and 25 females), from different Romanian regions. Genetic tests: performed in the Pediatric Genetics Unit of Royal Manchester Children's Hospital (UK) and in the Molecular Biology Department, University of Medicine and Pharmacy Cluj-Napoca (Romania). Methods used: ARMS Multiplex, PAGE, ARMS Duplex, ASO, SSCP/Heteroduplex and direct sequencing of DNA. Results: The genotype structure was: $\Delta F508/\Delta F508$: 31.1%, $\Delta F508/\text{non } \Delta F508$: 22.2%, $\Delta F508/x$: 4.44%, x/x : 46.6% (x -unidentified mutation). The frequency of $\Delta F508$ mutation was 46.6%. A number of 15 non $\Delta F508$ alleles were also identified and their frequency analysis revealed correlations with different regions of the world: 20% of mutations being more frequent in Central and Eastern Europe, 33.3% of them being frequent in Western Europe or geographical areas far away from Romania (Canada, Israel). A number of 46.6% mutations were rare or sporadic, from which there were 2 new mutations, identified in our group and not yet presented by other researchers: 1717-2AG,

within the intron 10 and R735K within the exon 13. Conclusions: the frequency of 46.6% for Δ F508 mutation in our group is similar with that from the surroundings countries with majority Caucasian population; the complexity of genetic structure emphasizes the heterogeneous character of Romanian population; the great number of unidentified alleles as well as the new identified mutations reveals the character of specificity of some mutations. (Study performed through a Grant Research Programme- CNCSIS/ A/1188/2004)

P1235. Analysis of VDR, COL1A1, CALCR and ER genes polymorphisms in the elderly people

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The polymorphisms of four genes (*TaqI* - VDR, *Sp1* - Col1a1, *AluI* - CALCR and *XbaI*, *PvuII* - ER) associated with some multifactorial diseases (osteoporosis, arthritis, endometriosis, mioma and others) were studied by PCR-RFLP in two groups of people from the northwest region of Russia. The middle age people group included 120 (60 men and 60 women - group 1) unrelated individuals of middle age (25-45), the group of elderly included 147 people (29 men and 118 women - group 2) unrelated individuals older than 70 years.

We have not elucidated any authentic differences in frequencies of genotypes and alleles of VDR, Col1a1, CALCR and ER genes between group of elderly people and population ($p > 0.05$) (tab 1). According to present data it might be speculated that group 1 and group 2 have not any inherited advantages for longer survival of people.

Tab 1. The frequencies of genotypes and alleles of VDR, Col1a1, CALCR and ER genes					
Genes	VDR (TT, Tt, tt & T, t)	COL1A1 (SS, Ss, ss, & S, s)	CALCR (TT, TC, CC & T, C)	ER (XX, Xx, xx & X, x)	ER (PP, Pp, pp & P, p)
middle age people, n=120					
Genotypes, %	42.18 42.86 14.96	62.59 36.05 1.36	56.46 34.01 9.53	13.61 39.46 46.94	25.85 51.02 23.13
Alleles, %	63.61 36.39	80.61 19.39	73.47 26.53	33.33 66.67	51.36 48.64
elderly people, n=147					
Genotypes, %	45.30 42.74 11.96	70.84 28.33 0.83	58.33 32.5 9.17	12.93 43.97 43.10	23.27 48.14 27.59
Alleles, %	66.67 33.33	85.00 15.00	74.58 25.42	34.91 65.09	47.84 52.16

P1236. Issues concerning familial inheritance of Hypospadias

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Hypospadias is a congenital malformation with a complex etiology which consists in abnormal opening of penile urethra. We have studied 756 cases of hypospadias in our clinic between 1975-2000. This trial was statistically significant. In order to determine the role of genetic factors familial study were performed to investigate the frequency of such malformation in relatives. An increase frequency could be explained by the transmission of an enzymatic defect in the synthesis of testosterone or decrease sensitivity of testosterone receptor. In 13.7% of cases significant familial aggregation of hypospadias (2 or more members of the same family) were identified. In 3.17% of cases related malformations were found (skeletal and cardiac). In 13.49% of cases hypospadias was associated to other genital malformations. The study determines the role of genetic factors in the etiology and pathology of hypospadias. The inheritance of hypospadias is probably polygenic multifactorial.

P1237. Association analysis of the dopamine transporter (DAT1) -67A/T polymorphism with bipolar disorder.

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An imbalance in the dopaminergic system in humans has been hypothesized to contribute to the pathogenesis of a number of psychiatric illnesses, including bipolar disorder, schizophrenia, and attention deficit hyperactivity disorder. We performed a case/control study on the DAT1 (HUGO approved symbol SL6A3) gene core promoter polymorphism -67A/T to analyze the possible association of either allele of this polymorphism with bipolar disorder. 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% [c2=10.3, df = 2, OR = 2.15 (95% CI 1.34-3.47, p ≤ 0.006]. The T-variant 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 for the contribution of the DAT1 gene core promoter polymorphism to the etiopathophysiology of bipolar disorder 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.

P1238. Consanguinity and Infertility

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Introduction: Consanguineous marriage is widespread in many parts of the world. There is an increased incidence of both congenital malformations and other conditions such as infertility which will present later. To assess the incidence of consanguineous marriage in Yazd province and its role in infertility.

Material and Methods: We studied 5200 married defined couples in 260 randomized clusters that were divided equally in ten different areas of the province including rural and urban areas. These couples were interviewed based on using a structured questionnaire to ascertain the prevalence of consanguinity, infertility and related epidemiological findings and assessed for etiological factors.

Results:

Our data showed that there were consanguineous marriage in 2153 couples (41.4%) (CI 95% from 41.2% to 42.7%) in different degrees including degree 2 (0.04%), degree 3 (27.3%), degree 4 (7.4%), degree 5 (6.7%).

In total, 276 cases of infertility were encountered and the overall prevalence of infertility was about 5.3% (CI 95% from 4.7% to 5.9%). In infertile couples, 135 cases (48.9%) had consanguineous marriage (P=0.009; Odds Ratio=1.38; CI 95% from 4.7% to 5.9%).

Conclusion:

Consanguineous marriage in couples was related to expression of infertility, but this wasn't significantly different in female and male factors infertility.

This study can assist physicians and genetic counselors to realize the incidence of consanguineous marriage in Iran and its role in expression of infertility and setting priorities for first level prevention of it.

P1239. The Genetic differentiation and Alu insertion polymorphisms (ACE, TPA25, PV92, Ya5NBC27, Ya5NBC148) in the Kazakhs Population of Middle Asia

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The Middle Asia are an interesting regions for studying relative influence of linguistic variability and geographic barriers on the genetic structure of populations. Polymorphic Alu-repeats occur in non-coding regions

and are thought to be evolutionarily neutral and present the objective markers for human population genetics and evolution studies.

Five Alu insertion polymorphisms were examined in three rural Kazakh populations of Middle Asia, represent to Turkic-speaking ethnic groups. A total of 152 unrelated healthy Kazakh people were studied. A portion of genes (ACE, TPA25, PV92, Ya5NBC27, Ya5NBC148) from genomic DNA was amplified by PCR and analyzed on a 6% polyacrylamide gel. The distribution of the empirical genotypes and allelic frequencies of Alu-polymorphisms was completely conformed to theoretical deviation of Hardy-Weinberg ($\chi^2=0.253$; $p>0.05$). The allele frequency distribution patterns were characterized by relatively high level of genetic diversity ($H_e=0.475$). The index of genetic differentiation (G_{st}) in three rural Kazakh populations was 0.0179. The highest estimate of genetic differentiation was at PV92 loci ($G_{st}=0.042$) along with relatively low G_{st} at the Ya5NBC27 loci -0.0007. The coefficient of genetic differentiation in the three populations studied was 0.012 (G_{st}) to relatively low level of genetic subdivision of the Kazakh populations. The frequencies of genotypes and allelic variants of Alu- polymorphic genes in Kazakh population are intermediate between mongoloid (Chinese, Mongols) and classical Caucasians. Phylogenetic analysis demonstrated close genetic relationship between the Kazakh, Uigurs and Uzbek ethnic populations.

It was confirmed, that the analysis of Alu insertion polymorphisms are tools for reconstructing ethnogenetic history of populations

P1240. Endothelial nitric oxide synthase gene polymorphisms and early human development

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Experimental data in mice and previously published results in humans point to a crucial role of NO in the course of pregnancy. It is known that variant alleles of the endothelial nitric oxide synthase (eNOS) gene are associated with increased susceptibility to pregnancy disorders and fetal defects. Some polymorphisms in the eNOS gene are also implicated in the development of age-dependent diseases. The objective of the investigation was to explore the probable association between the promoter region -C691T, intron 4 VNTR and 7 exon G894T of the NOS gene polymorphisms and human spontaneous abortions. A case-control study of the prevalence *NOS₃* gene polymorphisms were determined in the DNA samples from 166 embryos that had been spontaneously aborted between the 5th and 16th week after conception, and 188 adult controls. Preliminarily, the embryonic tissues were analyzed by cytogenetic methods and picked up the samples with normal karyotype only. The genotype frequencies for the G894T polymorphism was found to be out of Hardy-Weinberg equilibrium ($\chi^2=3.98$; $P<0.05$) due to heterozygotes excess ($h_0=0.46$, $h_e=0.40$). The frequency of C allele -C691T polymorphism among the spontaneously aborted embryos and the control group were 91.6% and 81.4%, respectively (OR=2.48; $P=0.0004$). The distribution of genotype frequencies was significantly different between the study and control groups for CT heterozygotes of -C691T polymorphism (13.3% and 35.5% respectively; $\chi^2=20.01$; $P<0.0001$). The results of the study indicate that the embryonic *NOS₃* 691CT genotype may protective affects the fetal viability in early human development.

P1241. Genetic structure of Eurasian populations based on Alu insertion data

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A total of 1049 individuals from Volga-Ural region of Russia (Turkic speaking Bashkirs, Tatars, and Finno-Ugric speaking Komis, Maris, Mordvins, and Udmurts), Central Asia (Turkic-speaking Kazakhs, Uzbeks, and Uighurs), the North Caucasus (Turkic speaking Karachays, Kumyks, Kuban Nogays, and Karanogays), and Siberia (Turkic speaking Yakuts, Tungusic speaking Evenks, Mongolic speaking Kalmyks) were analyzed using eight Alu loci (ACE, ApoA1, PV92, TPA25, NBC27, NBC102, NBC148, and NBC182). All loci were polymorphic in all populations. Genetic differentiation in various regions of the world was fairly substantial (using additional data of

Watkins et al., 2003 for the same Alu loci). Basing on 8 Alu loci data G_{st} value for the world dataset was 0.090. The highest level of genetic differentiation is observed in East and Southeast Asia (0.083), which is very close to the world's value. The lowest value can be seen in Central South Siberia (0.001). Principal component analysis showed that all of the Volga-Ural populations are plotted close to European populations. Populations of Central Asia and Central South Siberia forms rather distinct cluster together with Karanogays separating from populations of East and Southeast Asia. In conclusion, populations of the two boundary regions between Europe and Asia (Volga-Ural region of Russia, and populations of the North Caucasus) are more similar to European populations than to Asian populations. The North Caucasus populations demonstrated genetic pattern which is very close to Near East populations. European populations reflect neither geographic nor linguistic relationships. Siberian and Central Asian populations demonstrate more similarity and close genetic relationships.

P1242. The study of the STR polymorphism THO1 and CSF1PO loci in Volga-Ural populations

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In recent years, 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. Moreover on carrying out of medico-legal statistics is important to choose representative population correctly.

The polymorphism of STR loci THO1 and CSF1PO were studied in seven Volga-Ural populations: Bashkirs, Tatars, Chuvashes, Komies, Mordvinians, Udmurts and Russians. The distribution of the obtained genotypes did not deviate from Hardy-Weinberg equilibrium ($P>0.05$). Comparison of the informativeness of loci THO1 and CSF1PO allows us to consider the first more informative by findings of statistical parameters of forensic importance: the average value of the observed heterozygosity (H) was 0.794 in THO1 and 0.734 in CSF1PO; polymorphism information content (PIC) and power of discrimination (pD) were also higher in THO1 (PIC=0.747, $pD=0.896$) than in CSF1PO (PIC=0.691, $pD=0.880$); matching probability (pM) - 0.101 and 0.121, consequently; mean exclusion chance (W) - 0.544 in THO1, 0.467 in CSF1PO; the coefficient of allelic variety was 4.584 in THO1 and 3.810 in CSF1PO; index of Shannon diversity - 0.336 and 0.271, consequently.

In conclusion, investigated THO1 and CSF1PO loci can serve as highly informative markers for genetic research, forensic casework and determination of biological relatedness of individuals.

P1243. Genotoxicological monitoring of nurses handling cytostatic drugs evaluated before and after taking antioxidant support

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Purpose: The aims of this study were to evaluate genotoxic damage in nurses professionally exposed to cytostatics, and to investigate whether antioxidant treatment could influence reparation of genome damage. The influence of confounding factors, such as age, smoking status and duration of occupational exposure were also investigated.

Methods and patients:

Biomonitoring was performed on fifteen oncology unit nurses before and six months after taking commercial antioxidant preparation (OLIGOGAL®-SE). Fifteen unexposed healthy volunteers served as a control group. The sister chromatid exchange (SCE) assay, standard cytokinesis-block micronucleus (MN) assay, and proliferating rate index were used for evaluation of genotoxic damage. There were seven nonsmokers and eight smokers among exposed workers, and eight nonsmokers and seven smokers among control group.

Results: Significantly higher values of SCE and MN before (SCE

p<0.0001; MN p<0.05) and six months after antioxidant treatment (SCE p=0.001; MN p<0.05) were found in nurses group compared with control. Six months after antioxidant treatment SCE (p=0.001) and PI values (p<0.1) were found to be significantly decreased.

Analyses related to the smoking habits revealed significant difference in SCE value (p<0.1) between smokers and nonsmokers in the group of nurses.

Age and duration of occupational exposure did not significantly influence any of the genotoxicological parameters.

Conclusion: Results of this study showed that handling with cytostatic drugs may cause genome damages in occupationally exposed nurses and that antioxidant support may contribute in reparation of these genotoxic damages. Study also confirmed that smoking represents confounding factor in genotoxic risk

P1244. Preliminary results of the study methylenetetrahydrofolate reductase enzyme polymorphism (C677T) as maternal risk factor for Down Syndrome among Russian women

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Down's syndrome is the commonest chromosomal anomaly with an incidence of about 1:700 live births. This syndrome is a complex genetic and metabolic disorder attributed to the presence of three copies of chromosome 21. The extra chromosome derives from the mother in 93% of cases and is due to abnormal chromosome segregation during meiosis (nondisjunction). Except for advanced age at conception, maternal risk factors for meiotic nondisjunction are not well established. The relationship between chromosomal nondisjunction leading to aneuploidy and folate metabolism has drawn attention in the recent years. Several studies suggested that abnormal folate metabolism and the C677T polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene may be one of the maternal risk factors for Down syndrome. In order to confirm this association, we studied the prevalence of the C677T mutation in 56 mothers who had a child with Down syndrome and 54 control mothers with healthy children. The incidence of the mutant T allele in study group (29%) was the same as in control women (34%) $P = 0.51$. *MTHFR* C677T genotype frequencies were not significantly altered in mothers of children with Down syndrome ($P = 0.65$). Our preliminary results do not support the presence of an increased risk of Down syndrome in mothers carriers of the T allele in Russian population. Further research on larger samples is needed to test our results.

P1245. Y chromosomal STR haplotypes in Iranian population

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As both the Y chromosome and mitochondrial DNA show uniparental inheritance they are particularly useful for tracing the separate ancestry of paternal and maternal lineages in human populations. Y chromosome markers and their genetic diversity can be used to give information on male-specific patterns of migration and the origin and diversity of specific populations. Haplotypes based on polymorphic markers are easily constructed because of the lack of recombination in these genomic regions.

Desirable characteristics of markers which should be used are to have a high level of polymorphism in the population, and the lowest possible incidence of recurrent mutations. The variance in microsatellite marker has been used for population genetic study. The microsatellite mutation rate is 10-3, and the rate of those on the Y chromosome has been estimated to be 2.1x 10-3 in each generation. Furthermore, it has been found that Y chromosome variants tend to be more localised geographically than those of mtDNA and the autosomes. Putting all these data together, the Y chromosome is good to study as it shows paternal/clonal inheritance, and has an effective population size that is only 25% that of the autosomes.

For this study 8 of these widely used Y-chromosome markers Dys425, Dys426, Dys434, Dys435, Dys436, Dys437, Dys438 and Dys439 has been employed to analyses almost 300 males from 21 populations in Iran. These markers showed a good efficiency to discriminate between different ethnic groups.

P1246. Mitochondrial DNA polymorphism in three population samples of Tuvinians

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To study mtDNA polymorphism in Tuva Republic (South Siberian region of Russian Federation), HVS-I sequencing and restriction haplogroup confirmation was performed in three population samples of Tuvinians: Bai-Taiga administrative area (west of the region, N=169), Todja area (north-east, N=130) and Shinaan area (south-east, N=147). In total, 90 different HVS-I lineages were revealed, belonging to 17 different haplogroups, and West Eurasia-derived haplogroups encompass 7.5% of all mtDNAs. Low frequency of interethnic marriages in Tuvinians suggests that most of "European" lineages are not due to recent admixture. The most abundant and heterogenous haplogroup in Tuvinians is haplogroup C, which encompasses about one-half of their mitochondrial gene pool. More than 1% frequency estimates were obtained for haplogroups A, B, D, F, G, H, J, U and for 22 HVS-I lineages in total, but in particular areas these lineages were not always the same. Only 5 haplogroups and 4 haplotypes had more than 5% frequencies in total sample. 29 of 90 haplotypes were common with previously studied Tuvian sample (Derenko et al. 2003) whereas only 8, 10 and 14 haplotypes were shared with Yakuts, Altaians, and Buryats, respectively. Analysis of migrations has shown that the genetic diversity in Todja population increased due to migrants from other areas of Tuva who brought 17 new haplotypes into this area population. Such effect has not been shown for Bai-Taiga and Shinaan populations. According to the results, Tuvinians constitute quite isolated and slightly subdivided population, with high haplogroup C prevalence. The work was supported by RFBR grant 04-04-48792.

P1247. Friedreich spinocerebellar ataxia in Romania

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Introduction: Friedreich spinocerebellar ataxia is an autosomal recessive neurodegenerative disease, characterized by progressive evolution, pyramidal cerebellar phenomena, suppression of osteotendinous reflexes and deep sensitivity of lower limbs, ataxic gait, dysarthrias, cardiomyopathies, slow speech, dysmetria associated with hypermetria, diabetes mellitus.

Material: We investigated 142 patients with Friedreich spinocerebellar ataxia admitted to the „Horia Radu” Center of Neuromuscular Pathology, Vâlcele, Covasna county.

Methods: history, clinical examination, family inquiry, genetic record, reconstruction and analysis of pedigrees of the families included in the study; the biostatistical method, which allowed us to establish the frequency of the heredo-collateral history of Friedreich ataxia, the sex distribution of the cases included in the study, the frequency of Friedreich ataxia among the cases of the Center of Neuromuscular Pathology, as well as the incidence of the disease in the different Romanian counties.

Results: The analysis of pedigrees confirms the autosomal recessive inheritance of Friedreich ataxia; only 38.1% of patients presented a heredo-collateral history of Friedreich ataxia; the study of sex distribution shows that 45.1% of cases were females and 54.9% males; the distribution of cases according to the onset age shows a 58.2% frequency in the age group 10-15 years; the cases of Friedreich ataxia represent 7.03% of all cases of neuromuscular diseases admitted in the same period: the highest incidence of Friedreich ataxia was found in Brasov, Sibiu, Bihor, Timis, Botosani counties.

Conclusion: The incidence of this disease can be reduced by the detection of carriers of gene X25, prenatal diagnosis by DNA analysis and genetic counseling.

P1248. Molecular genetic studies of sporadic spinocerebellar ataxia (SCA) in the cyprriot population

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Spinocerebellar ataxias (SCA) form a heterogeneous group of neurodegenerative disorders that primarily affect the spinal cord,

the cerebellum and brainstem. Autosomal dominant and recessive inheritance has been detected in families and sporadic cases have also been reported. The main clinical features include gait difficulties, ataxia and dysarthria. The age of onset is usually within the 4th decade. Several associated features may present in different patients that include optic atrophy, peripheral neuropathy, retinal degeneration, and dementia. Several attempts to clinically classify SCA patients have proved to be unsuccessful due to the overlapping features seen within the same SCA type and in many cases within the same family. Many SCA loci and genes have recently been identified that prove the extensive genetic heterogeneity of this group of degenerative disorders. The most frequent mutation is a CAG repeat expansion, which causes a polyglutamine expansion at the protein level. Fifty sporadic patients that clinically conform to the diagnosis of SCA have been identified in the Cypriot population. Due to the high prevalence of FRDA in the western part of the island we initially analysed the above patients at the FRDA gene. All patients were negative for the FRDA expansion. SCA patients were then analysed for a mutation in the SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12, SCA17 and DRPLA genes. No expansion has been detected. We, therefore, conclude that SCA in the cypriot population is not caused by any of the most frequent SCA mutations detected in other populations.

P1249. Decreased frequencies of ABCA1 polymorphisms R219K and V771M in Hungarian patients with coronary heart disease and stroke

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Genetic polymorphisms of ABC-transporter A1 (ABCA1) may alter the regulation of plasma HDL-level, promoting or protecting from arteriosclerosis. Recently, several common SNPs have been recognised in ABCA1, but their effects on protein-function or their role are still controversial.

We investigated 244 unrelated, randomly selected patients with ischemic stroke, 150 patients with coronary heart disease (CHD) and 193 blood-donors for allele frequencies (AF) of three common ABCA1-polymorphisms (R219K, V771M, I883M). Compared to control (30.8±4.7%; 4.9±2.2%), decreased AF were found in both patient groups for R219K, and V771M (stroke: 28.7±4.1%; 3.1±1.6%, CHD: 25.7±5.0%; 1.3±1.3%). Upon stratification by age of onset, more pronounced AF-decreases were observed. In a subset of stroke patients younger than 50, both variants occurred in significantly lower frequencies (22.4±5.5%; 1.8±1.7%). Similarly, among CHD-patients younger than 60, AF of R219K and V771M (22.6±7.5%; 0±1.6%) decreased. V771M was almost exclusively (35/36) found in individuals carrying the R219K allele. The allele frequency of the third variant, I883M did not show significant differences in the groups and subgroups studied. Our data confirm earlier observations that ABCA1 R219K and V771M polymorphisms may be associated with a protective role against CHD and extend those to another important pathologic condition namely stroke. These protective effects seem to be more pronounced in subsets of patients with younger ages.

P1250. Malignant course of hypertrophic cardiomyopathy in India

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Hypertrophic cardiomyopathy (HCM) is a heart muscle disorder exhibiting pronounced phenotypic variation in clinical features ranging from benign to malignant forms and with high risk to heart failure and sudden death. HCM is familial in majority of cases and is transmitted as an autosomal dominant trait. Sudden death in HCM can be initiated by several different triggers which include ventricular arrhythmia, atrial fibrillation, myocardial ischemia, abnormal vascular control and autonomic dysfunction. The aim of the present study was to identify the percent of sudden death associated with the subtypes of HCM and

the possible mechanisms leading to sudden deaths on a preliminary data.

95 confirmed cases of HCM were considered in the present study of which 43 had obstructive type of HCM. Our study revealed an increased preponderance of the condition among the males (3.5:1) when compared to previous studies. Of the 95 probands, 3 died due to HCM in a follow up of 2 years. The family history of sudden death was found to be 7% among non-obstructive HCM and 14% among obstructive HCM. This frequency of 21% (pooled) is very high compared to 12% sudden death reported from earlier studies. Further the history of syncope was also found to be double among the obstructive HCM cases compared to the non-obstructive form. In conclusion, autonomic dysfunction and abnormal vascular control could be acting as possible independent triggers of sudden death in obstructive HCM, whereas hypotension and myocardial ischemia could be playing a role in non-obstructive HCM related sudden deaths.

P1251. Are ACA- and GGC-divergent MJD haplotypes the signature of de novo mutational events?

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The autosomal dominant neurodegenerative Machado-Joseph disease (MJD) has been reported worldwide in families from very distant origins. A previous haplotype study encompassing the expanded (CAG)_n region within the *MJD1* gene and three intragenic biallelic markers has shown the ACA and GGC haplotypes in 94.4% of the 72 informative MJD families, collected from 15 different countries. In order to clarify whether other expanded chromosomes with haplotypes different from these two most common are the evidence of different MJD mutational origins, or just the result of recombination and/or recurrent mutations at analysed biallelic markers, we have (1) extended the SNP-based haplotype study, genotyping three additional markers; (2) calculated gene diversity indices based on four flanking microsatellites, less than 250 kb apart from the (CAG)_n repeat, and (3) allele-specifically amplified and sequenced a 4 kb region from expanded chromosomes of 8 MJD patients with the AGA, GGA and still undisclosed (A/G)GC haplotypes. Newly studied SNPs did not discriminate additional MJD lineages, although, in some cases, complete haplotypes were not assessed due to the observed heterozygosity. Results from microsatellites' typing in these eight patients have, however, shown the highest molecular diversity (0.44) when compared to that found in the rest of the analysed chromosomes carrying either ACA (0.39; n=91) or GGC (0.25; n=61) haplotypes. Sequence differences were also observed throughout the 4 kb analysed region, although a comparison to the control data is still in course. Present results suggest that different MJD origins may be underlying the haplotypes other than the ACA and GGC.

P1252. Type 2 Diabetes mellitus and PPAR γ and CAPN-10 genes polymorphism in the Northern Greek population

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Type 2 diabetes mellitus is the most common form of diabetes worldwide, affecting approximately 4% of the world's adult population. It is multifactorial in origin, with both genetic and environmental factors contributing to its development. Genetic polymorphism in different genes has been associated with the disease in a number of populations.

Known polymorphisms in the genes coding for the peroxisome proliferator-activated receptor γ (PPAR γ) and the cysteine protease calpain-10 (CAPN10), were analysed in 92 patients with type 2 diabetes and 100 healthy individuals (control sample), all coming from Northern Greece.

The polymorphism Pro12Ala (exon B) in the PPAR γ gene was analysed by the PCR-RFLPs method. The results obtained suggest that the Pro12Ala polymorphism protects against type 2 diabetes

mellitus in the studied population and affects some clinical traits in diabetic patients.

Five single nucleotide polymorphisms, designated as SNP-19 (intron 6), SNP-43 (intron 3), SNP-44 (intron 3), SNP-63 (intron 13) and SNP-110 (exon 10) in the CAPN-10 gene, were analysed by PCR-RFLPs or MS-PCR method. The statistical analysis indicated that the genetic variation in the five CAPN-10 SNPs is not associated with type 2 diabetes mellitus in the studied population.

Additional studies based on a larger population group will provide a better understanding of the contribution that these genes make to diabetes risk in the Greek population.

P1253. Familial amyloid polyneuropathy V30M in Portugal: evolutionary dynamic of a disease in expansion

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Familial amyloid polyneuropathy (FAP) type I is an autosomal dominant disorder caused by mutations in the transthyretin (TTR) gene. With the exception of a few families, Portuguese focus is associated with the V30M mutation. FAP is highly prevalent in Portugal, where Andrade first identified it in 1939. We reviewed our register that includes 2029 patients observed between 1939 and December 2003. Of these, 1900 patients have (or belong to families with) molecular confirmation of V30M mutation. 70% of the patients (53% of the families) have been diagnosed in the last twenty years. Families were classified as 'de novo families' or 'previously undiagnosed families' according to absence or presence of similar disease in previous generations. Absence of the disease is due to incomplete penetrance, not to *de novo* mutations. *De novo* families predominates among recently diagnosed families (67% versus 42%). Age-of-onset (in yrs) is significantly significantly higher in index cases of *de novo* families (45.5 ± 11.8) than in index cases of undiagnosed families (32.1 ± 7.7). New families with a previously silent mutation are diagnosed every year. FAP is a disease in expansion in Portugal. The evolutionary dynamic of FAP gene is discussed.

P1254. New SNP in the exon two of gene encoding neutrophil elastase II in Iranian population

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The gene for human neutrophil elastase, a powerful serine protease carried by blood neutrophil and capable of destroying most connective tissue proteins, was cloned from a genomic DNA library of normal individual. Peripheral blood obtained from 30 normal individuals. Total RNA was isolated from fresh-polymorph-prep separated cells by using RNA standard techniques. RNA was analyzed by employing PCR amplification of reverse transcribed using a total of ten specific primers. Mutational analysis was performed by bidirectional sequencing methods. We amplified five exon of ELA2 gene separately and sequenced each exon. We have found new SNP in exon two codon number 44. It was a silent mutation C to A substitution but no changes in amino acid sequence Alanine has induced. The codon sequence was GCG that has been changed to GCA.

P1255. An investigation of the FRAXA intermediate allele phenotype using the ALSPAC cohort

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The FRAXA trinucleotide repeat at Xq27.3 gives rise to fragile X syndrome when fully expanded and both premature ovarian failure (POF) and fragile X tremor and ataxia syndrome (FXTAS) when in the premutation range. Reports of phenotypic effects extending into the intermediate repeat range are inconsistent but some studies suggest that these smaller expansions predispose to special educational needs (SEN). This study utilises the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort to investigate cognitive and behavioural

variables that might be associated with FRAXA intermediate alleles. The current study fails to find any strong evidence of association of FRAXA intermediate alleles with SEN, behavioural problems or cognitive difficulties. However, we have found some suggestive evidence of an association between Pervasive Developmental Disorder (PDD) and intermediate alleles. Our findings illustrate some of the difficulties encountered in identifying individuals with SEN. The power to identify specific components of cognitive and behavioural difficulties was reduced due to elective drop-out which is characteristic of longitudinal studies. Our results demonstrate the non-random loss of participants from this cohort and highlight problems that may arise when such data are used in genetic association studies.

P1256. Frequencies of the C598T mutation in VHL associated with autosomal recessive erythrocytosis

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Familial benign polycythemia (OMIM 263400) is a rare autosomal recessive disorder that is characterized by erythrocytosis, normal leukocyte and platelet counts, and increased erythropoietin production. There are more than 100 patients who are Chuvash by nationality and lived in northern-east part of the republic.

Ang et al (2002) identified substitution C598T in homozygous state in polycythemic Chuvash. This substitution lead to amino-acid Arg200Trp change in VHL gene in all individuals affected with Chuvash erythrocytosis.

We investigated DNA from 34 patients and 40 healthy relatives from 14 families. All patients are homozygous in respect to C598T mutation in VHL gene. Moreover we examined DNA from 422 healthy unrelated Chuvash. 14 heterozygous and 1 homozygous carriers were among them. So allelic frequency of C598T mutation is 2% for Chuvash, and frequency of the disease in Chuvash population must be 1:2500.

We investigated presence of C598T mutation in neighbouring populations and Russians. We discovered 6 heterozygous carriers among 344 Mary (allelic frequency 1%, calculated disease frequency 1:10 000), 2 heterozygous carriers among 221 Udmurts (allelic frequency 0.5%, calculated disease frequency 1:40 000). There was no C598T mutation among 271 Russians.

Individuals affected with Chuvash erythrocytosis discovered in Chuvash population. Affected individuals among Mary population did not discover in spite of relatively high calculated frequency of erythrocytosis. This can be explained by benign course of the disease, that clinicians did not always identify, or by modifier genetic factor that present in Chuvash population.

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P1257. Heritability of complex traits in an Italian genetic isolated population.

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The molecular bases of complex and quantitative traits can be better identified studying genetically isolated populations. The aim of present study is to investigate heritability of the most common complex and quantitative traits in Carlantino, a small village from Southern Italy. Data from 1,417 individuals were collected and used. Traits include blood pressure, height, weight, body mass index, waist to-hip-ratio, uric acid, cholesterol, triglycerides, glucose, Na, K, Cl, Mg, P, LDH, CPK, alkaline phosphatase, AST, ALT, bilirubine, blood formula. The variance component method implemented in SOLAR was used to estimate heritability (h^2) that represents the total additive genetic heritability after the effect of all covariates has been removed. Covariates included were sex, age or any phenotypes (other than the trait), and/or interactions of these. After analyses traits with a highest heritability value were: Height (0.70, SE=0.05, $p<0.0001$) Triglycerides (0.69, SE=0.07, $p<0.0001$) Uric Acid (0.60, SE=0.03, $p<0.0001$), MPV (0.61, SE=0.01, $p<0.0001$), MCH (0.59, SE=0.02, $p<0.0001$), MCV (0.56, SE=0.09, $p<0.0001$); these three last traits as expression of the significant proportion of thalassemic cases in the population. Absence of any statistical significance was obtained for creatinine, alkaline phosphatase, gammaGT, and WHR (Waist to-hip-ratio). In conclusion,

our study is the first report on heritability of such large number of traits in a genetically isolated population. Our findings suggest that a substantial part of variance of some traits is explained by genetic factors, thus helping us on focusing the search for corresponding genes on those traits with a strongest heritability component.

P1258. The load of hereditary pathology in Tomsk, Russia

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Medical genetic study of the population of Tomsk, Russia had been performed. The population sample comprised 412 720 subjects. For each nosological group, the loads of Mendelian pathology with different modes of inheritance had been determined. 71 autosomal dominant diseases had been found in a total of 454 patients, with hereditary syndromes being the most prevalent. Autosomal recessive pathology was represented by 69 diseases found in 376 patients, hereditary syndromes being the most prevalent too; and X-linked pathology, by 11 diseases in 89 patients. The prevalence rate had been calculated for each nosological form. The loads of autosomal dominant, autosomal recessive and X-linked diseases were 1.48 and 1.35 per 1000 people and 0.66 per 1000 men. The spectrum of hereditary pathology in Tomsk population was described. Migration was the major factor influencing the prevalence rates of hereditary pathology.

P1259. Analysis of *GSTM1* and *GSTT1* genes polymorphisms in newborn, middle age and in elderly people from the North-West region of Russia

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The polymorphisms of metabolic genes (*GSTM1*, *GSTT1*) responsible for xenobiotic conjugating enzymes of Phase II detoxification system were studied by PCR in three groups of people from the North-West region of Russia. The newborn group consisted of 57 children born in Ott's Institute - group 1, the middle age cohort included 83 unrelated individuals of middle age (25-45) - group 2, the group of elderly people were represented by 147 unrelated individuals elder than 70 years. The frequencies of *GSTM1* 0/0 genotypes were similar in all three groups (45.7%, 45.8%, 44.0%, respectively). Low frequency of null allele homozygotes for *GSTT1* gene in the group 1 (14%), middle (18%) - in the group 2 and rather high (28%) in the group 3 were found. The difference in the frequencies of null allele homozygotes between the newborns and elderly people was significant ($\chi^2=3.96$, $p<0.05$). The frequencies of null alleles of both *GSTT1* and *GSTM1* genes were increased, but not significantly, in the group 3 (13.6%) compared to group 2 and group 1 (9.6%, 8.7% respectively). It might be speculated that people homozygous for null allele of *GSTT1* gene passes some metabolic advantages which support their longer survival.

P1260. Familial aggregation of common forms of migraine in a group of Portuguese families

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Migraine is a common neurological disorder, with a complex mode of inheritance. Migraine with aura (MA) and migraine without aura (MO) are the two most common forms of this disease. Several studies have described an increased risk in relatives of migraineurs and suggest that genetic factors may be implicated in both subtypes. We studied familial aggregation of MA and MO in 171 Portuguese families of probands with MA, MO or both. Relatives were diagnosed according to the criteria of the International Headache Society.

Familial aggregation was evaluated by estimating the relative risk of the disease, first in the total number of relatives and then in relatives, according to their degree of relationship to the proband. Risk in the general population has been estimated previously at 8.8%. We also estimated the risk for spouses to disentangle the influence of genetic and environmental factors in migraine.

Relatives	Risk Estimate (Odds Ratio)
Total	4,68
Parents	5,60
Siblings	7,28
Children	7,86
Grandparents	3,35
Uncles	4,61
Cousins	3,99
Nephews	2,06
Spouses	1,08 (not significant)

Odds ratio was significant for all types of relatives and increased with degree of kinship to the proband, whereas the risk for spouses was not.

These results point out to familial aggregation in our sample and suggest a genetic component involved in the two common subtypes of migraine.

P1261. Investigation deletion *GSTT1* gene in Ukraine

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Glutathione S-transferase T1 (*GSTT1*) gene ensure the synthesis of the relevant enzyme product, that belong to the Phase II detoxification system responsible for biotransformation and degradation electrophilic xenobiotics. For this gene known nonfunctional null allele (0), which is formed as a result of extended deletion.

GSTT1 null genotype is known as a risk factor predisposition to lung and bladder cancer as well as to some other environmentally induced diseases such as chronic bronchitis, bronchial asthma, alcoholic cirrhosis.

Analysis *GSTT1* null genotype was performed by PCR in the group of 71 healthy volunteers from Ukraine. The frequency of the *GSTT1* null genotype is 21% ($n = 15$). This frequency is comparable to population controls obtained in studies for population of the UK (61/325, 19%) and USA (20/98, 20%) but it is higher than Russian population (7/72, 9.7%).

The result of our research can be the background for genetics testing and consulting individuals from chemical pollutant area of Ukraine and for precise therapy of patients with high risk of development *GSTT1* associated pathologies.

P1262. Advances in association mapping of disease genes using linkage disequilibrium maps.

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We have developed a simple yet powerful approach for disease gene association mapping by linkage disequilibrium (LD). This method is unique since it applies a model with evolutionary theory that incorporates a parameter for the location of the causal polymorphism. The method is based on single marker tests and LD maps, which describe the pattern of LD by assigning a location in LD units (LDU) for each marker. As a proof of principle, we tested our method using 27 SNPs that cover an 890 kb region flanking the *CYP2D6* gene with known location. Four functional *CYP2D6* polymorphisms cause the poor drug metabolising phenotype. Previous LD mapping studies using single markers and haplotypes have identified a 390 kb region associated with this phenotype. Using a metric LDU map, the commonest functional polymorphism within the gene was estimated to be located only 14.9 kb from its true location, surrounded within a 95% confidence interval of 172 kb. Using same modelling procedure, the kb map had a relative efficiency of 33% compared to the map in LDU. Our findings show that the support interval and location error are smaller than any published results on these data. Despite the low resolution and the very strong LD in the region, our results provide evidence of the substantial utility of LDU maps for disease gene association mapping. These tests are robust to large numbers of markers and are applicable to haplotypes, diplotypes, whole-genome association or candidate region studies.

P1263. Interparalog gene conversion patterns of HBII-52 C/D box snoRNAs cluster at 15q11-q12

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Brain expressed, paternally imprinted C/D box HBII-52 snoRNAs are organized in a ~99 Kb cluster of 47 copies on chromosome 15q11-q12. They are suggested to post-transcriptionally modify 5HT2C receptor transcripts by affecting the (A) to (I) editing and/or alternative splicing. The HBII-52 copies share high homology with each other (>98%), and over half of them are expected to be functionally active. Due to high level of sequence identity, gene conversion is anticipated to occur between snoRNA homologues.

In order to study the patterns of gene conversion of the HBII-52 snoRNAs cluster we have analysed the DNA variability of 23 presumably functional copies in 70 Spanish individuals. Our preliminary data indicate that the snoRNAs are characterized by high nucleotide diversity, high overall gene conversion rates for the region and multiple gene conversion hot-spots. In 40 chromosomes analysed so far we have identified 31 SNPs, 20 of which are located at potential gene conversion sites with tract length of 5 bp or more. Four gene conversions between homologues that involve more than one paralogous sequence variant (PSV) were detected, with gene conversion tracts between 6 to 31 bp. We have detected HBII-52 snoRNAs haplotypes that are marked by several gene conversion events and have accumulated minor alleles for multiple SNPs.

Multiple SNPs are found at the proximal and distal parts of the snoRNAs cluster while central copies are devoid of variants suggesting that some homologues might represent gene conversion hot spots.

P1264. Human von Willebrand factor gene STR polymorphism (vWFII) in Russian, Belorussian and Kalmyk and Yakut populations.

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An STR polymorphism in Human von Willebrand factor gene (vWFII) possess high individual discrimination power and have an application in medical, forensic and population genetic studies. The variability of this DNA marker have been studied in different ethnic groups of Eastern Europe: Russians (Arkhangelsk and Kursk regions), Belarussians (Woodland region), Kalmyks (Elista region) and Yakuts (Central region of Sakha Republic). Allele typing was performed using the PCR and subsequent electrophoresis followed by silver staining. Nine alleles of the locus were noted in populations studied. Allele frequency distributions are in agreement with Hardy-Weinberg expectations in all populations. The level of observed heterozygosity was high and varied from 0.65 to 0.79. In contrast with other hypervariable STR markers studied in these populations, observed allele frequency distributions in distant ethnic groups from Eastern Europe show no statistically significant difference, whereas there is significant differentiation for Yakut population from Asia. This may reveals conservative fitness action on the gene, because of the gene product importance for survival.

P1265. Population structure of Dagestan mountain isolates based on Alu insertion and Y chromosome data

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Mountain inhabitants of Dagestan speak North Caucasian languages and are known to be characterized by extreme linguistic diversity in a relatively small geographic area. In addition to small population size most ethnic groups tend to live isolated. 8 Alu insertion polymorphisms (PV92, ACE, APOA1, B65, A25, NBC27, NBC123, and TPA25) were typed in a total of 390 unrelated individuals from 7 autochthonous populations of

Dagestan. Populations of Avars, Chamalals, Bagvalals, Andis, Lezgis, Dargwasses and Tabassarans inhabiting mountain part of Dagestan were analyzed. The overall pattern of the MDS analysis suggests that mountain isolates of Dagestan like other Caucasus populations clustering with West Eurasian populations being closer to Middle East populations. A large proportion of Y chromosome haplogroups defined using 46 NRY biallelic markers are those considered to have originated in the Middle East. It has been shown earlier based on mtDNA and Y chromosome variation analysis that mountain inhabitants reveal great distinction which is in good agreement with the language differentiation pattern of the region. But what regards autosomal variation, estimates based on our Alu data set shows extremely low genetic differentiation ($F_{ST} = 0,008$). In general lower values compared to those obtained by uniparentally inherited markers are expected but the amount of difference is somewhat striking possibly explained to some extent by marker characteristics itself. Anyway autosomal variation reflects shared genetic background suggesting recent common ancestral population in the past. Contemporary genetic structure has been primarily shaped by genetic drift.

P1266. Glucokinase mutations in women with gestational diabetes mellitus selected by clinical criteria

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Maturity- onset diabetes of the young (MODY) is a genetically and clinically heterogeneous form of non- insulin dependent diabetes mellitus, characterized by early onset, usually before 25 years of age, an autosomal dominant inheritance and primary defect in insulin secretion. Mutations in at least six genes have been shown to underline MODY, including GCK mutations (encoding glucokinase). Glucokinase-related MODY 2 is a common form of this disorder, especially in children with mild hyperglycemia and in women with gestational diabetes and a family history of diabetes. The purpose of this study was to estimate the prevalence of MODY 2 in Polish gestational diabetic patients. Selected gestational diabetic women fulfilled the following criteria: age<35 years, BMI< 25, a small increment (<3 mmol/l) during 2-h oral glucose tolerant test and a history of type II or gestational diabetes in a first or second-degree relatives. The PCR- SSCP analysis involving coding regions of the 12 exons and the intron-exons boundaries of the GCK gene was used. In 12 probands (10%) of the 119 patients we detected two novel GCK mutations: G448fsinsG, E312Q, two previously reported S383L, Y215Y and four intronic variants: IVS2-12C>T, IVS3-8G>A, IVS4+26C>A, IVS7-13A>G. All mutations were absent from 120 control subjects. We found previously reported polymorphism IVS9+8C>T. The frequency of the C and T alleles was respectively 0.8 and 0.2. The obtained results indicated on relatively low prevalence of MODY 2 in Polish gestational diabetic patients. This work is supported by Ministry of Sciences and Informatics grant no PO5E09326

P1267. Polymorphism of the MTHFR gene and serum lipid levels in Serbian child population

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As it is known thermo labile polymorphism C677T of the methylenetetrahydrofolate reductase (MTHFR) gene has been shown to result in increased total homocysteine concentrations caused by a decreased enzyme activity. Homocysteine could act as inhibitor or promoter of oxidation LDL cholesterol.

The aim of this study was to determine C677T genotype in 609 (310 boys and 299 girls) healthy children and investigate a possible association among the TT genotype and serum lipid levels. C677T polymorphism was genotyped by PCR amplification of specific fragment of genomic DNA followed by digestion with the Hinfl restriction enzyme. We have found that genotype was wild type CC in 260 (42, 7 %), heterozygous CT 268 in (44, 0 %) and homozygous mutant TT in 81 (13, 3%) children. Total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were determined for each MTHFR genotype group (CC, CT, and TT) in boys and girls. LDL cholesterol are significant higher ($p<0, 02$) in TT

group of children. Also, in boys levels of both total and LDL cholesterol among the CT and TT groups differed significantly ($p<0.02$, $p<0.05$). We did not find any significant association between the homozygous genotype (TT) and HDL cholesterol and triglycerides level in both sexes.

P1268. mtDNA hypervariable regions in determination of local human populations diversity after war resettlement

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Comparison between the genetic data sets collected before and after the war proved significant influence of war in Bosnia and Herzegovina 1992-1995 on genetic structures of local human populations.

MtDNA hypervariable regions HV1 and HV2 diversity of three local human populations in alpine area of B&H was investigated. Molecular diversity indices and genetic structure parameters were calculated for three subdivided populations based on their spatial isolation and effects of war resettlement. Because of its developed infrastructure and connections with urban area, one of the investigated villages Dejcici is the center of refugees' repatriation after four-year exodus. Present population of this village includes returnees that originate from several other villages in the same mountain area. Populations of the two other villages Bobovica and Lukomir were expelled and returned home following the seize of atrocities. The named populations were compared against standard Bosnian population.

Usefulness of mtDNA hypervariable regions in determination of local human population after the war resettlement was tested using different diversity measures as well as with adapt specific population genetics measures. Comparison with result of short tandem repeats analyses of the same populations have been done in order to determine the level of differences of these genetic markers for using in determination of local human populations diversity.

P1269. Cross-Population Mapping Genes for Schizophrenia-Spectrum Disorders in Daghestan Genetic Isolates

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Schizophrenia presents across a wide clinical spectrum, and a large number of genes are presumably involved. Genetic isolates may be used to reduce the genetic and clinical heterogeneity of these complex schizophrenia-spectrum disorders. A genome-wide linkage scans for schizophrenia susceptibility loci in pedigrees from Daghestan genetic isolates provided highly significant evidence of cross-population differences in linkage patterns. We present the findings of a parametric linkage analysis of families from four primary and secondary genetic isolates. We found strong evidence for linkages with schizophrenia-spectrum disorder at two regions of chromosome 22 (22q11, with maximum heterogeneity $\alpha=1.00$ and a LOD score of 8.7, and 22q13, with $\alpha=0.75$ and a LOD score of 6.7). Furthermore we confirmed a locus on chromosome 17 (17p11.1-p12) with a LOD score of 5.03 ($\alpha=1.00$) that replicated earlier reported our results in a single pedigree. These findings should provide sufficient rationale for subsequent fine mapping and positional cloning efforts at these loci in order to elucidate the underlying susceptibility genes.

P1270. Real-time profiling of miRNAs from single cells

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A new miRNA quantitation method has been developed using stem-loop primers for reverse transcription (RT) followed by real-time TaqMan®. A total of 240 miRNA assays were designed and tested using as few as single cells or as little as 25 pg total RNA. The C_T values correlated ($R^2 = 0.999$) to the copy number over seven orders of

magnitude. The expression of miRNAs varied greatly from 0 to 32,091 copies per cell in mouse tissues. Presence of genomic DNA did not affect the miRNA quantitation. The assays discriminated between two miRNAs that differed by as little as a single nucleotide, and between mature miRNAs and their precursors. This method allows accurate and sensitive miRNA expression profiling, uncovers precise changes of miRNA expression during stem cell differentiation, and identifies potential miRNA markers specific to particular cell type and cancer. Comparison of miRNA expression profiles between normal and cancer tissues will be presented.

P1271. Sub-classification and molecular characterization of early stage breast carcinoma using Applied Biosystem Human Genome Survey Microarrays

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DNA microarray technology has shown great promises in deciphering molecular phenotypes of breast cancer. In this study, we profiled gene expressions on 20 biopsy tissues of early stage breast carcinoma using Applied Biosystem's Human Genome Survey Microarrays. Two main previously defined clinically relevant subtypes of breast tumors, Luminal A (longest survival time) and Basal (shortest survival time) were identified. Statistical analysis identified 1210 genes as signature genes characterizing the two subtypes of breast cancer. Panther™ functional classification and biological pathway analysis on these signature genes depicts a more detailed molecular portrait of these expression-based subtypes: Signature genes of Luminal A subtype were over-represented by biological function/processes such as cell structure and amino acid metabolism, while genes over expressed in the Basal subtype were over-represented by oncogenes, cell cycle signaling pathways.

In an attempt to identify the best set of genes as potential biomarkers for sub-classifying breast cancer, the same 20 patients samples were analyzed using Stanford cDNA Arrays and Agilent Human Whole Genome Arrays. The results from the three microarray platforms were found to be highly correlated. Using PAM analysis, a minimal numbers of genes were selected to best characterize and distinguish the Luminal A and Basal subtypes of tumors. These classifier genes were further verified by TaqMan assays. Validated by multiple gene expression platforms, the classifier genes identified in this study defined potential prognostic molecular markers for breast cancers.

P1272. High-resolution molecular characterization of 16p13.3 and 11p15.5 rearrangements causing alpha- and beta-thalassaemia by multi-color 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. 85% of cases). Rearrangements in the beta-globin gene cluster on 11p15.5 account for approx. 10% of all beta-thalassemia mutations and for the majority of Hereditary Persistence of Fetal Hemoglobin (HPFH) syndromes. The molecular tests commonly used to identify deletion types of alpha- and beta-thalassasemias and HPFH at present are gap-PCR, Southern blot analysis and Fluorescent In Situ Hybridization (FISH) analysis. However, the applicability of these techniques is limited to known deletions, dependent upon the hybridization probes available and may involve 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 to detect rearrangements in the alpha- and beta-globin gene clusters. MLPA allows the precise quantification of up to 51 probe sequences within a nucleic acid sample using a single tube assay. We describe the design of two sets of 35 and 51 probes, covering a region of 700 kb of the alpha- and 250 kb of the beta-globin gene cluster respectively, amplified by primers

labeled with up to three different fluorophores. Besides the detection of the most common deletions, several alpha- and beta-thalassemia causing deletions, not previously described, were found using this assay. Because of its robustness and simplicity, this technique should become the standard for the detection of (large) rearrangements causing hemoglobinopathies and other diseases in laboratories capable of performing automated DNA fragment analysis.

P1273. The influence of neuropeptide 'Semax' on the synthesis of neurotrophic factors in rat retina and brain

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Glaucoma is a group of optic nerve progressive neuropathies which is followed by the morphological alterations of the optic nerve disk. The main elements damaged under this disease are ganglion cells of retina. Glaucoma is characterized by different microcirculatory changes, ischemia of the retina and neuron death. Intranasal introduction of 'Semax' is used in ophthalmologic practice for the treatment of glaucoma. According to the one of hypothesis 'Semax', the physiologically active analogue of adrenocorticotrophic hormone (4 - 10), stimulates the production of neurotrophic factors BDNF and NGF in the glial cells of the neural tissue and increases the vital capacity of the neurons. We observed its influence on the expression of BDNF and NGF in rat retina and some parts of brain under the different types of the peptide introduction. The PCR analysis showed that under the subconjunctival introduction 'Semax' caused the increase of the mRNA expression of neurotrophic factors in the retina. Under the intranasal introduction the level of the expression of BDNF and NGF mRNA decreased for 40 % in the retina. This level of BDNF mRNA increased for 30% in hippocampus under such conditions. Expression of NGF mRNA in hippocampus and visual region of cortex increased for 40% and decreased for 20% in thalamus. We hypothesized those therapeutic effects of 'Semax' as intranasal medication was not directly connected with the activation of neurotrophic factors synthesis in retina.

P1274. Universal Primer APEX: a flexible and large-scale genotyping platform

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Our purpose is to work out universal primer APEX and then greatly increase throughput and precision of large scale genotyping, while reducing cost. In the present project we will attack several limiting key factors for such analyses, including design oligonucleotide primers, parallel analyses of large sets of genetic markers, and array-based genotype scoring platform. These technologies are expected to enable whole-genome association studies for mapping candidate genes for common diseases.

We use restriction and universal adapter ligation procedure for amplification of target-DNA from genomic DNA. Before PCR with universal primer we reduce genomic DNA complexity on highly specific affinity array. In this step we hybridize probe-specific DNA to on an array oligonucleotides and remove unbound material during washing step. After elution of hybridized DNA from bound oligonucleotides, we run PCR with universal primer and primer extension on array.

Our ultimate purpose is to work out high-throughput and flexible genotyping platform that uses a one universal primer assay to genotype 15,000 SNPs per individual on a single oligonucleotide array.

P1275. Expanding the scope of molecular analysis

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Recently, a new set of tools has emerged that simplifies certain aspects of molecular testing and provides additional benefits. Called MultiCode® (for multiple coding), these tools evolve around the idea that genetic material is not limited two base pairs. Indeed other base pairs could exist chemically and one additional base pair now does exist commercially. The components required to advance the commercialization potential, such as triphosphates for enzymatic incorporation of this base pair have recently become available for isoguanine (iG) and 5'-methyl-isocytosine (iC). This presentation will discuss current and potential areas of interest for this base pair

as they pertain to human genetic testing. Specifically, two platform technologies have recently emerged using iC and iG; MultiCode RTx for solution based quantitative analysis and MultiCode PLx for solid phase multiplexed end-point analysis. Together, these two platform technologies will be applicable to some +90% of all genetic testing. This presentation will focus on our experience using the MultiCode PLx for human genetic detection. The presentation will discuss reports using a highly multiplexed PLx assay for analysis of the CFTR gene and using lower complexity PLx assays to target various other human genes developed as "home brews".

P1276. *In silico* identification of transcription factors involved in cellular differentiation by promoter analysis of co-regulated genes

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Having analysed changes in gene expression during the differentiation of human primary skeletal myoblasts on microarrays, we aimed for the identification of the transcription factors responsible for the observed changes in gene expression. To this end, upstream sequences of co-regulated genes were retrieved and aligned with 322 recognition sequences of vertebrate transcription factors (TFs). The majority of differentially expressed genes included in the search had not previously been associated with myogenesis. Still, we observed that their promoters were significantly enriched in regulatory elements binding myogenic TFs, such as MEF2, SRF and Msx, when compared to control promoter sequences or promoters from genes with unchanged expression. This demonstrates the power of the algorithm in the identification of relevant TF binding sites. In addition, we found that promoters of up-regulated genes were significantly enriched in binding sites for TFs that have not or have only recently been implicated in myogenesis. Among these are Forkhead transcription factors and the PBX/MEIS1 heterodimer. Their binding sites were also significantly over-represented in the homologous mouse promoter sequences, further substantiating their functionality. Binding sites for mentioned transcription factors are often found together in the same promoter. These clusters of binding sites can be used to predict involvement of genes not measured on the microarray in myogenesis. The approach developed can be extended to expression datasets coming from other cell models, and will be helpful to distill candidate TFs responsible for the observed co-regulation of gene expression.

P1277. Comparative use of 10-mer DNA oligonucleotides and DNA:LNA oligonucleotides for sequence analysis by arrayed primer extension

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The ability of DNA microarrays to measure thousands of binding interactions simultaneously has led to their rapid adoption in many applications. One opportunity is to synthesize a universal array containing all 4^N possible oligonucleotides of length N. Due to the potential complexity of the large number of different oligonucleotides on chip, it is difficult to find optimal reaction conditions. One way to stabilize A:T and G:C-rich duplexes is to incorporate chemically modified nucleotides in short universal primers.

The aim of current study was to test 10-mer oligonucleotides for sequence analysis by arrayed primer extension (APEX) method in microarray format on glass slides. In the next stage we investigated the use of modified Locked Nucleic Acid (LNA) monomers in constitution of 10-mer APEX primers at different positions. Theoretically addition of a single LNA base in DNA/RNA oligonucleotide can raise Tm value of the duplex by 4 - 9°C.

Contrary to the expected rise of APEX signals the effect of inclusion of LNA monomers into DNA oligonucleotides resulted in significant deterioration of the signals of APEX reactions. The hybridization experiments performed in microchip format revealed either no effect or higher thermostability of modified oligonucleotides compared to the DNA tenmers. Inclusion of LNA monomers into DNA oligonucleotides probably interferes in DNA polymerase reaction with chimeric oligonucleotides.

From the results it can be concluded that the incorporation of LNA monomers in short oligonucleotide primers is perspective for hybridisation based microarray methods but not effective for APEX reaction based methods.

P1278. ATM Gene Mutations Detection, Haplotype Analysis, mt-DNA and D-loop Variation in Iranian Patients with Ataxia-Telangiectasia.

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Ataxia-Telangiectasia is an inherited autosomal recessive disorder characterized by defect in a number of distinct organ systems. Symptoms include progressive cerebellar ataxia, telangiectasia, immunodeficiency, chromosomal instability radiation sensitivity and increased incidence of malignancies. The ATM gene of human chromosome 11q22.3 has been identified as the gene responsible for human recessive disease ataxia-telangiectasia. ATM is encoded in 66 exons and spans 150kb of genomic DNA. In this study 20 families with at least one affected child with clinically suspect for ataxia-telangiectasia were examined and extracted DNA was amplified by using standard methods.

Three exons which were hot spot for point mutations in ATM gene were detected by PCR-RELP and SSCP. The polymorphic bands sequenced to detect the possible point mutations. In this manner, mt-DNA was tested by 6 primers for existence of any mitochondrial deletions. We also amplified and sequence the D-loop of these patients by standard sequencing techniques. Likewise four molecular markers: D11S2179, D11S1787, D11S535, D11S1343 were genotyped in A-T families. Those markers were amplified using extracted sequence primers from Gene Bank. The amplified products were separated using denaturing PAGE gels, and the data were analyzed to detect their pattern of inheritance in each family. We have found three mutations (insertion, substitution) in the examined exones and mtDNA deletions including 2 individuals with 7.5kb deletions, one with 5kb together with a 9.0kb deletion for all. The samples were then sequenced to admit deletion breakpoints. Also the results of the D-loop variations related to A-T patients have been discussed in the presentation.

P1279. Identification of p53 genotype among Iranian Ataxia-telangiectasia (A-T) patients

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Recent studies showed an association between the specific diseases and single nucleotide polymorphisms (SNPs). Ataxia-telangiectasia (A-T) is a cancer-prone and radiation-sensitive syndrome. It is also, a recessive multi-system disorder caused by mutations in the ATM gene at 11q22-q23. The risk of cancer is substantially elevated in A-T patients. It is reported that the ATM/p53 signalling pathway is altered by a very low ATM expression or by the presence of a mutated p53. In the absence of ATM, humans show a primary immunodeficiency that includes low serum antibody titers.

To investigate the relationship between p53 codon 72 polymorphism and risk for AT, we collected samples from AT patients and healthy population from different region of Iran with different ethnicity groups (Fars, Mazandaran and Turk). The p53 Pro72Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and direct DNA sequencing analysis in 207 healthy controls and 19 AT patients. Among the patients and healthy subjects with Fars, Mazandaran and Turk ethnicity, the genotype frequency of p53 Pro72Arg were 15.8% and 34.8% for Arg/Arg, 73.7% and 45.9% for Arg/Pro, 10.5% and 19.3% for Pro/Pro, respectively. Significance differences were found for p53 allele distribution among patients and healthy individuals. Our finding suggested that the frequency of Arg allele is more than healthy Iranian population.

P1280. Investigation of NQO1 genotype polymorphisms between Iranian healthy population and Ataxia-telangiectasia (A-T) patients

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Ataxia-telangiectasia (A-T) is a cancer-prone and radiation sensitive syndrome. The A-T patients are hypersensitive to radiation, free radicals are generated by radiation. The NAD(P)H: quinone oxidoreductase (NQO1) enzyme prevents redox cycling which leads to generation of free radicals. It has been reported that this gene has a single nucleotide polymorphism (SNP) at site of codon 187 (nucleotide 609). A Pro to Ser substitution at codon 187 of the NQO1 gene is associated with a loss of NQO1 protein and enzyme activity. To investigate the relationship between NQO1 codon 187 (nucleotide 609) polymorphism and risk for A-T patients. We collected samples from AT patients and healthy population from different region of Iran with different ethnicity groups (Fars, Mazandaran, Turk). The NQO1 Pro187Ser genotypes were determined by polymerase chain reaction - restriction fragment length polymorphisms (PCR-RFLP) for 206 healthy control and 20 AT patients. We found only 20 Iranian A-T patients. Among the patients and healthy controls (with Fars, Mazandaran and Turk ethnicities) the genotype frequency of NQO1 Pro187Ser were 70% and 67% for Pro/Pro, 25% and 28.2% for Pro/Ser, 5% and 4.8% for Ser/Ser, respectively. No significance differences were found for NQO1 genotype distribution among patients and healthy individuals. Our finding suggested that NQO1 genotype does not play an important role in developing Ataxia-telangiectasia.

P1281. Application of an integrated Bayesian approach to the detection of copy number changes in arrayCGH images.

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Bayesian methods have been widely applied to data analysis problems in the life sciences. Their application to copy number change detection has however been limited to the post processing of primary data extracted from microarray images by traditional black box image processing techniques. This combination of Bayesian and black box approaches is sensitive to variability; both from experimental and operator sources in the primary data.

In this presentation we describe a novel approach which combines the three major stages of arrayCGH analysis; extraction of primary data, normalisation and copy number change detection, in a single integrated solution. The Bayesian model based approach is introduced as a means of improving the measurement of the underlying biological quantities while at the same time estimating the likely variability associated with those measurements. The importance of a single statistical framework as a means of carrying forward knowledge of this variability through to the detection of copy number changes is illustrated by examples of how detected copy number changes may be qualified by confidence estimates; meaningful within the clinical context.

The presentation will also illustrate how an integrated Bayesian approach removes operator intervention from the analysis of arrayCGH data thereby making this powerful technology accessible to individuals without a strong background in microarray image analysis and the associated mathematical techniques.

P1282. Deletions of the SHOX gene in patients with Leri-Weill dyschondrosteosis evidenced by the MLPA approach.

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The human SHOX gene (Xp22 - Yp11.3) is involved in the pathogenesis of diseases characterized by the presence of short stature, being rearranged in up to 70% of patients with Leri-Weill dyschondrosteosis (LWD) and in up to 12% of patients with idiopathic short stature. In the majority of cases, the SHOX gene alterations consist of large deletions, detectable by FISH or molecular analysis of intragenic CA repeats. However, FISH is a low throughput technique and is able to detect only large deletions, and analysis of CA repeats can be performed only when DNA of the parents of the proband is available. In recent years, the Multiple Ligation Probe Amplification (MLPA) technique has demonstrated to be a useful tool in the study of deletions of several genes. In this study we used the MLPA approach for the detection of

SHOX deletions in 14 patients affected by LWD (9 of which previously analysed by FISH) and 20 with idiopathic short stature (15 of which previously analysed by FISH). MLPA analysis evidenced SHOX deletions in 6 LWD patients (43%), and in no case with idiopathic short stature. In all cases, MLPA analysis confirmed results previously obtained by FISH. Interestingly, MLPA analysis showed two alternative proximal breakpoints, producing two different deletions of 2.3 Mb and 500 kb, respectively. These results demonstrate that MLPA analysis is an useful tool in the study of SHOX gene, showing a reduced labour intensity as compared to FISH and providing more information about the breakpoints of the deletion.

P1283. Genomic organization and classification of conserved non-coding sequences in vertebrates reveals distinct classes of genes

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The increasing number of available sequenced genomes is enabling researchers to determine with increasing confidence the presence of conserved non-coding sequences. We have focused our efforts on understanding the evolution and the genomic organization of these regions relating them to the function and structure of neighboring genes comparing five vertebrates genomes in two phyla.

We have mapped CNSs to the exon-intron structure of the genes and classified each analyzed gene on the basis of CNS localization and GO classification. We have also attempted to cross phylum boundaries by analyzing the fragmentation of mammalian CNSs in fish.

Preliminary results suggest that the density of conserved sequences is higher in introns with respect to flanking regions in most of the analyzed genes. Furthermore CNSs seem to be present in higher number in the first intron within the coding region as compared to other introns, and this seems to be related to an increase in intron size.

Our GO classification suggests that the minority of genes that show a higher number of CNS in the flanking regions has a statistically significant enrichment for genes involved in development and transcription, suggesting a different regulation mechanism for this class of genes. Moreover genes without CNSs (but conserved in all five vertebrates) show a statistically significant enrichment for involvement in biosynthesis and metabolism indicating a much simpler regulation mechanism. Moreover we show that it is important to take into account fragmentation of non-coding sequences when comparing them across longer evolutionary distances.

P1284. Rapid mutation detection method of complex genes by Heteroduplex Analysis with Capillary Array Electrophoresis

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Scanning for mutations in complex genes in a large number of samples is hampered by the large size of these genes and the scattering of mutations all over their coding sequences. We have transferred Heteroduplex Analysis (HA) by Conformation Sensitive Gel Electrophoresis (CSGE) of the two major breast cancer predisposing genes, BRCA1 and BRCA2, to a multicapillary DNA sequencer in order to increase the throughput of this technique. This new method, that we have called Heteroduplex Analysis by Capillary Array Electrophoresis (HA-CAE), is based on the use of multiplex-PCR, different fluorescent labels and heteroduplex analysis in a 16-capillary DNA sequencer. To date, a total of 114 different DNA sequence variants (19 insertions/deletions and 95 single nucleotide substitutions -SNS-) of BRCA1 and BRCA2 have been successfully detected by HA-CAE. In addition, we have optimised the multiplex-PCR conditions for the colorectal cancer genes MLH1 and MSH2 in order to analyse them by HA-CAE. Both genes have been amplified in 13 multiplex groups which contain the 35 exons and their corresponding flanking intronic sequences. MLH1 and MSH2 have been analysed in nine Hereditary Non-Polyposis Colorectal Cancer (HNPCC) patients, and we have found six different DNA changes: one complex deletion/insertion mutation in MLH1 exon 19 and another five SNS. Only the complex mutation and one SNS may be classified as cancer-prone mutations. Our experience has revealed that HA-CAE is a simple, fast, reproducible and sensitive method to scan the sequences of large multi-exon genes.

P1285. Elaboration and usage of point mutation detection technology based on AS-PCR using automated DNA analyzer.

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We developed a new method of point mutation detection based on allele-specific PCR using capillary electrophoresis for PCR-product analysis. It consists of a reaction with three primers where two of them are allele-specific and third is common primer. Allele-specific primers with different length of 5'-terminal sequences are used for development of the AS-PCR products with different lengths that allow us to detect a heterozygote. Length standards are used for exclusion of false results and for increasing method reliability. The standards are special synthesized DNA fragments lengths of that are intermediate between AS-PCR product lengths. In case of capillary electrophoresis with fluorescent detection the length standards and the PCR fragments are labeled with different fluorophores and analyzed in the same capillary. The elaborated technology was used for development of the tests for detection of mutations in several genes which are responsible for thrombosis: C677T mutation in MTHFR gene, Leiden mutation in factor V gene and G20210A mutation in prothrombin gene. Also the method was used for detection of M235T, T174M and G(-6)A mutations in angiotensinogen gene, which are responsible for essential hypertension and for detection of Sp1 (Colla1), BsmI (VDR), XbaI and Pvull (ERα) polymorphisms concerned with osteoporosis.

The elaborated technology is appropriate for clinical usage and large-scale polymorphisms analysis.

P1286. A new approach to argue causality of FBN1 mutations in UMD-FBN1

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Mutations in the fibrillin-1 gene (FBN1), the major component of extracellular microfibrils, are associated with Marfan syndrome (MFS), but also with a spectrum of conditions phenotypically related to MFS called type-1 fibrillinopathies. To facilitate mutational analysis at the molecular level and to provide the tools to search for genotype/phenotype correlations, a Locus Specific DataBase UMD-FBN1 was created in 1995. Among the 711 mutations indexed, the global analysis revealed two classes of mutations. The first one (40.89%) corresponds to mutations predicted to result in shortened fibrillin-1 molecules: nonsense mutations, splicing errors, insertions, duplications and deletions. The second one (59.11%) corresponds to missense mutations. Since in vitro validation of these nucleotide variations is not possible in diagnostic settings, indirect arguments must be accumulated to define if these missense mutations are really causative. In this context, we have annotated the FBN1 sequence for Highly Conserved Domains (HCD). These data list for a given position the known arguments such as: cysteines implicated in disulfide bonds, amino acids implicated in calcium-binding, N-linked glycosylation, furine/Pace sites, metalloproteases sites, RGD, or very conserved amino acids of unknown function in cb EGF-like, TGFβP, EGF-like modules, as well as in fibulin-like (Cterm) and in LTBP-like (Nterm) modules newly described. The new annotation of the FBN1-HCDs shows that 83% of missense mutations are localized in one of these HCDs even if numerous parts of the protein remain to be functionally annotated. Thus up to 90% of all FBN1 mutations can be considered as pathogenic, even in the absence of validation.

P1287. Sequencing the short arm of human chromosome 21

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The sequence of the euchromatic portion of the human genome

is essentially complete. However, large regions of the genome, comprising ~5-7% of the total, remain to be sequenced. These regions include the short arms of the acrocentric chromosomes (13, 14, 15, 21 and 22). The acrocentric chromosomes are of great interest as they are involved in many translocations causing human genetic disease. Human chromosome 21 has special significance because of its involvement in Down syndrome. The sequence of the short arm of Hsa21 (21p) would thus be important not only for understanding chromosome 21 genetics and disease, but an important step in the characterisation of these unexplored regions of the genome and toward the completion of the human genome project. We have constructed a BAC library containing human sequence from only Hsa21 and shown that it contains clones from 21p. So far we have generated approximately 1.3 Mb of new sequence from 21p (estimated to be 10-15% of the total), which shows that 21p contains regions which have the characteristics of euchromatic sequence. Gene prediction by EST and in silico based methods, followed by confirmation of ~30% gene models by rtPCR in 24 human tissues, indicates the presence of many expressed sequence on 21p. In this project, we aim to produce a sequence contig of 21p and characterise 21p with respect to genes and repeat content.

P1288. Anti-iNOS locus in the human genome: structure and potential role in natural antisense RNA mediated regulation of iNOS expression

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Recently we have demonstrated that DNA inversions, mutations involving major rearrangements of the genome and usually regarded as catastrophic to gene function, can have an important role in the creation of novel genes (Korneev & O'Shea, *J Mol Biol Evol*, 2002). We suggested that these genes form a special class of genetic elements capable of producing antisense RNA molecules. Specifically, using a molluscan model system we have shown that nitric oxide synthase (NOS)-related antisense RNAs can function as specific negative regulators of NOS gene activity. Our experiments indicate that this novel mechanism of gene regulation is important for learning and memory formation (Korneev et al., *J Neurosci*, 2005). By employing computational methods of analysis we have started our search for similarly organised elements in other species including human. Here we report on our discovery of a locus in the human genome whose structure appeared to have been affected by an internal DNA inversion. This region of the genome has the highest similarity to the gene encoding inducible isoform of nitric oxide synthase (iNOS). Furthermore, by analysing the human EST databases, we have identified a transcript produced from this locus. We refer to this transcript as anti-iNOS RNA. As expected the anti-iNOS RNA contains a region, which exhibits approximately 90% complementarity to iNOS mRNA and therefore it can be considered as an example of natural *trans*-encoded antisense RNA. Moreover, by using RT-PCR we confirmed that the anti-iNOS locus is transcribed in the human brain.

P1289. Some 10% of L1-mediated retrotranspositions are associated with significant genomic deletion in humans

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Analysis of L1 elements engineered for retrotransposition in cultured cells has revealed that ~6% of L1 retrotranspositions resulted in significant deletions of genomic DNA (Gilbert et al. and Symer et al. *Cell* 2002). The frequency of L1 retrotransposition in humans has been estimated to be between 1/2 and 1/33. If sizable deletions were to accompany even 1-5% of L1 retrotranspositions *in vivo*, the cumulative effect upon genome structure over evolutionary time would be very substantial. An analysis of recently integrated L1Hs Ta elements in the

human genome has suggested that the frequency of DNA deletions created upon L1 retrotransposition is 3.4% at the most (Kazazian and Goodier, *Cell* 2002; Myers et al. *AJHG* 2002). However, "even the youngest of the polymorphic Ta sub-family L1s in the draft human genome sequence must be thousands of years old". An analysis of recently integrated Alu sequences concluded that only ~0.8% (2/244) of Alu retrotranspositions were associated with genomic deletions (Salem et al. *MBE* 2003); but the first case appears to occur through gene conversion whereas the second would have occurred through double-stranded DNA break repair. It is potentially more instructive to analyze insertions that have occurred into mammalian genomes in the past 100 years. However, no deletions were found to be associated with the 14 original pathological L1 insertions examined (Kazazian and Goodier 2002). Here we reported that some 10% of L1-mediated retrotranspositions are associated with significant genomic deletion in humans through a meta-analysis of all reported L1-mediated events causing human genetic disease.

P1290. Investigation of mutations involved in macular corneal dystrophy in Iranian patients

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Macular corneal dystrophy (MCD) is an inherited autosomal recessive disorder, which is clinically characterized by progressive corneal stroma clouding in both eyes.

MCD is subdivided three Immunophenotype (MCD types I, IA and II), some mutations in the carbohydrate sulfotransfase 6 genes (CHST6) were identified to cause MCD.

The CHST6 genes mapped on chromosome 16q22. That encoded a protein with the same name.

DNA was extract from 17 blood samples from suspected of MCD patients. PCR method was used to amplified regions three common MCD point mutations which is hot spot for this disease.

Sequencing method was used to identify new point mutations.

We found new mutations in some patients that none of these mutations was detected in the control group.

P1291. The Cypriot and Iranian National Mutation databases

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The exponential discovery rate of new genomic alterations, leading to inherited disorders, as well as the need for comparative studies of different populations mutation frequencies necessitates recording their population-wide spectrum, in online mutation databases. The National Mutation Databases are continuously updated mutation depositories, which contain extensive information over the described genetic heterogeneity of an ethnic group or population. Here, we report the construction of the Cypriot (<http://www.goldenhelix.org/cypriot>) and Iranian National Mutation databases (<http://www.goldenhelix.org/iranian>), both derived from an academic effort to provide high quality up-to-date information on the underlying genetic heterogeneity of inherited disorders in the Cypriot and Iranian populations respectively. Both databases have been built and maintained online using the specialized ETHNOS software (Patrinos et al., 2005) and contain brief summaries of the various genetic disorders included within each database. 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 these countries. Furthermore, numerous links to the respective Online Mendelian Inheritance in Man (OMIM) entries and, when available, to the locus-specific databases fruitfully integrate the databases content into a single web site. Both databases can serve as valuable online tools for molecular genetic testing of

inherited disorders in these countries and could potentially motivate further investigations of yet unknown genetic diseases in the Cypriot and Iranian populations.

P1292. Human gene MOB, a member of yet unknown evolutionarily conserved gene family, codes for sphingomyelin synthase.

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Prior investigation of human brain cDNA libraries revealed an evolutionarily conserved gene *MOB* that has been cloned in silico on chromosome 10. Analysing hypothetical primary structure of the transmembrane peptide product proposed for the *MOB* major transcript we have found it to be identical with the newly described primary structure of human sphingomyelin synthase 1 (SMS1); now we consider *MOB* as a gene encoding SMS1. In human, two yet uncharacterized genes paralogous to *MOB* (SMS1) were revealed. These genes termed par1 *MOB* and par2 *MOB* are localized on chromosomes 4 and 10, respectively; together, they represent a novel gene family. *MOB*, p1 *MOB* and p2 *MOB* orthologs of conserved genomic structure were found in warm-blooded animals; orthologs of p2 *MOB* were also found in *D. melanogaster* and *C. elegans*. We have examined *MOB* (SMS1) transcription activity; expression levels of the major *MOB* transcript (transcript encoding SMS1) were assessed among different human tissues by means of semi-quantitative RT-PCR. Comparing to the maximal expression level detected within the brain tissues, transcript abundance within kidney is one and a half times less, within lung and liver - two times less, within spleen and lymphatic node - seven times less. We have also analysed the expression pattern of the alternative *MOB* transcript lacking the longest coding exon VII and found it to be similar with that of the major transcript.

P1293. Structural organization of the human complexin 2 gene (CPLX2) and aspects of its functional activity.

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Using *in vitro* and *in silico* approaches we have characterized the organization of the human complexin 2 (CPLX2) gene. This encodes for a protein of 134 amino acid residues, contains five exons, is localized on human chromosome 5q35.3, and spans more than 87 kb. We performed *in silico* analysis of the CPLX2 5' untranslated region (UTR) and propose an alternative variant of the gene transcript. Compared to the mRNA reported earlier, this transcript bears a partly altered 5'-UTR associated with the same open reading frame. Both CPLX2 transcripts share exons III-V; the alternative transcript is devoid of exons I and II, and includes exon A instead. Exon A is localized within CPLX2 intron 2 about 7 kb upstream to exon III. Using reverse transcription polymerase chain reaction (RT-PCR) we detected both types of transcripts in human cerebellar mRNA. *In silico* data suggest that two alternative TATA-less promoter regions separated by 74 kb govern the expression of two CPLX2 transcripts. Several potential transcription start sites were detected by primer extension for each of two alternative CPLX2 transcripts. Activity of the promoter proximal to the translation start site is believed to be mediated by a GAGA-box. Analysis of extended CPLX2 transcripts revealed several 15-LOX-DICE-like elements as well as K-box and GY-box functional sites within their extended 3'-UTRs. These elements may be involved in the processes of stabilization and post-transcriptional regulation of the gene activity. Structurally, the organization of CPLX2 transcripts is conserved in human, mouse and rat.

P1294. Improvement of the sensitivity of DNA methylation analysis using the Pyrosequencing real time sequencing technology

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Pyrosequencing is a real-time sequencing technology that can overcome the tediousness of current DNA methylation analysis procedures when several CpGs are to be studied. Quantification of the methylation at each cytosine is conducted during the sequencing process by analysing the proportion of C and T (or A and G) nucleotide after bisulphite treatment.

Several reports have demonstrated the sensitivity and specificity of Pyrosequencing for quantifying SNPs. This capacity has been extended to methylation analysis of 2 to 6 successive CpGs in a single run. We sought to improve read-length of this technology to facilitate the mapping of differentially methylated regions (DMRs) in the genome. By using an improved enzyme mix and adding single stranded DNA binding protein to the reaction, we obtained reproducible results for as many as 10 successive CpGs in a single sequencing reaction spanning up to 80 nucleotides. A minimum amount of 10 ng of bisulphite treated DNA is necessary to obtain good reproducibility and avoid preferential amplification.

We applied the Pyrosequencing technology to analyse the methylation profile of 4 DMRs in the vicinity of the imprinted genes H19 and IGF2 in human lymphocytes and showed that reproducible variations of the methylation status of consecutive CpGs do exist in these regions..

Conclusion: Pyrosequencing is a reliable and straight forward technology for DNA methylation analysis with several improvements when compared to other existing procedures.

P1295. Towards automated provision of comparative positional candidates for deafness and blindness: Oxford grids and the comparative prediction of orthologues

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There are many inherited forms of deafness and blindness that have not yet been characterized at the molecular level. A powerful strategy for molecular characterisation of any inherited disorder involves the identification of comparative positional candidates from comparative maps.

Deafness and blindness are ideal disorders for comparative analysis as, at any rate in the young, most non-environmental forms are Mendelian recessives and are likely to block the same pathways in all mammals. While RNA analysis offers a more direct approach to candidate loci, it depends on biopsies which, for the eye or ear, are rarely ethical. Comparative mapping offers an alternative route to likely candidates.

Astute observations on the mouse, cat and dog, combined with induced mutation in the mouse, provide extensive comparative data for identifying the molecular basis of inherited forms of sight and hearing disorders in humans.

As a step towards automating the identification of comparative positional candidates, we have developed software for drawing electronic Oxford grids and for comparative predictions of orthologues. Grids involving cat, dog, human, mouse and rat, together with comparative predictions of orthologues, are viewable at <http://oxgrid.angis.org.au>.

P1296. Loci of recurrent segmental aneuploidy in the genome of healthy and mentally retarded subjects detected by Array-CGH

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Using a 3,700 BAC array we performed Array-CGH on over 100 children suspected of chromosomal disorders and some of their unaffected parents. Per individual we found on average statistically significant aberrant signals with 28 BACs. In 6% of our patients some of these signals represented pathogenetically relevant aberrations. In the remainder of our subjects these aberrant signals appeared independent of clinical symptoms and occurred with a frequency of 2% to 49%. In the patient and in the healthy subject subset of our study population the same BACs occurred with similar frequencies as loci of recurrent segmental aneuploidy (RSA). In total more than 500 BACs indicated loci of RSA, which covered in total 2.1% of the euchromatin. Approximately 25% of loci of RSA occurred as duplications only, 37% as deletions only, and 38% as both deletions and duplications. The

genomic distribution of loci of RSA varied widely among autosomes, with a preponderant location in the Giemsa-light, gene-rich bands. Approximately 30% of loci of RSA contained no genes at all, 8% contained only enzymes, and the remainder mixtures of genes from different classes, including numerous transcription factors. We conclude that loci of RSA occur frequently in the general population. Since loci of RSA segregated from healthy parents to affected children, we speculate that they confer no apparent clinical consequences, and may be neutral to evolutionary pressures.

P1297. Different CFTR genotypes induce dissimilar expression patterns in CF putative modifier genes

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Cystic fibrosis (CF; #219700) is a disorder caused by mutations in the CFTR gene. Although a strong genotype-phenotype relationship in CF has been observed, significant clinical variability exists among CF patients carrying the same genotype or the same class of mutations. While some of the variability is undoubtedly caused by environmental factors, genes other than CFTR likely modify phenotypes. Even if a certain number of putative gene modifiers have been investigated in CF, none are clearly substantiated.

We built an home made cDNA microarray containing a large number of genes involved with intracellular processes in cells where CFTR is normally expressed and genes occurring in cells that respond to abnormalities caused by CFTR defect (i.e. inflammatory, ion flux), to identify genes and pathways interacting or compensating CFTR functions. We studied the expression profiles of a total of 150 selected candidate genes in two different human epithelial bronchial-derived cell lines (CuFi1, F508del/F508del; CuFi3, F508del/R553X). We observed change in the expression in 40 genes (27%) of the examined active in the inflammatory response, in protein degradation, in molecular chaperone actions, in ion flux and in CFTR physical interaction. A few of these, shared the same expression pattern in the two cell lines (i.e. S100A8, S100A9, SMAD3, SMAD4, ENaC), whereas others showed a different expression pattern (AP1M2, AP3B1, AP1S1). These data demonstrate that different CFTR mutations cause diverse genomic response, and indicate strategies to develop therapeutic targets that may benefit specific patients with disease associated to a particular genotype.

P1298. High throughput experimental verification of predicted tissue and tumor specific splice isoforms

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Any failure or error in the splicing control mechanism can be involved in a number of pathological processes, such as cancer or neurodegenerative diseases. Therefore, splice isoforms that are disease specific could serve as excellent diagnostic markers, which are easily identifiable by PCR.

A computational prediction strategy was used based on the genomic mapping of EST consensus sequences and library annotation provided in the GeneNest database. Out of 427 genes with at least one tissue specific transcript as well as 1120 genes showing tumor specific isoforms, a subset of predicted isoforms were experimentally verified by an RT-PCR screening approach. An experimental strategy has been set-up that allows to screen expression of genes in 112 different human tissues of multiple developmental stages and cell lines. Within this project, the electrophoretic separation of RT-PCR products turned out to be the bottleneck impeding the switch from a medium to a high throughput strategy. To circumvent the limitations of slab gel analysis, a lab prototype of an automated on-chip electrophoresis system that allows high throughput analysis of DNA fragments was implemented in the workflow. In our experimental set-up, we analyzed RT-PCR samples on 4 x 96 well plates within a defined sequence of consecutive one-on-one measurements. The high throughput experimental verification of computationally predicted tissue specific isoforms revealed a high success rate in confirming their expression in the respective tissue.

The combination of computational prediction of alternative splicing events with high throughput experimental verification facilitates the efficient detection of tissue and tumor specific transcripts.

P1299. In vitro transfer of ceruloplasmin polypeptide into rat mitochondrial matrix

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Ceruloplasmin (Cp) is a key participant of copper and iron metabolism. There is a single copy of Cp gene in haploid genome, but its tissue-specific isoforms of transcripts and proteins were found at different stages of ontogenesis. Research is devoted to search of novel Cp isoforms in rat cells. Cp polypeptides were found in mitochondrial matrix of brain, liver, testicles and mammary gland, but not in heart and spleen. It was also shown that Cp isoform (mtCp), not sequestered into secretory pathway membranes, was synthesized in cytoplasm. In vitro [¹²⁵I]mtCp is transferred to mitochondrial matrix. It was registered based on the data of radioautography of immunoprecipitates, analyzed using SDS-PAGE. The transfer is ATP energy consumption process; it concentrationally depends on the presence of soluble factors of cytoplasm. Mitochondria swelling, valinomycin and oligomycin treatments, as well as removal of labile copper ions and copper ions of active centers from Cp molecule have no influence on Cp transfer. Both translocators of outer and inner mitochondrial membranes participate in the transfer, but [¹²⁵I]Cp is also successfully transferred to myoplasts, obtained after digitonin treatment. Computer analysis of rat chromosomal Cp gene revealed the existence of open reading frame (75 bp) with exon 3 in intron 2 and the upstream localized potential promoter. Predicted Cp-mRNA isoform corresponds to mtCp. RT-PCR analysis with specific primers for mtCp-mRNA detected the predicted isoform in liver and brain, but not in heart and spleen. Direct sequencing of PCR-product showed that mtCp-mRNA contained intron 2 region.

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P1300. Structural and functional characterization of the human SOX14 promoter

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SOX14 is a member of SOX gene family of putative transcriptional regulators implicated in the control of diverse developmental processes. Although SOX gene expression patterns suggest tissue- and development stage-specific control of gene expression, little is known about mechanisms responsible for expression of these genes. In order to elucidate the molecular mechanisms controlling the expression of the human SOX14 gene, we have determined the transcription start site using primer extension method and carried out the structural and functional analysis of the regulatory region responsible for its expression. Our result indicates that the transcription of the SOX14 initiates at the single major site at the guanine residue 251 bp upstream of the start codon, although the existence of a minor transcription start site can not be excluded.

To identify the DNA regions responsible for the control of SOX14 gene transcription we have analyzed the ability of truncated fragments from the 5' flanking region of the SOX14 gene to drive expression of CAT reporter gene. Functional mapping of the 5' regulatory region revealed that the sequence between -470 and +201 bp is essential for minimal basal activity of the SOX14 promoter.

We have identified that NF-Y transcriptional factor binds to the CCAAT box motif present in the regulatory region of the SOX14 promoter. By mutation analysis we have shown that CCAAT box motif present in the SOX14 promoter plays a functional role in the transcription of this gene.

P1301. Functional characterization of the human SOX3 promoter: Identification of transcription factors implicated in basal promoter activity

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Sox genes constitute a large family of developmentally regulated genes involved in the decision of cell fates during development and implicated in the control of diverse developmental processes. Sox3, an X-linked member of the family, is expressed in the brain from the earliest stages of development. It is considered to be one of the earliest neural markers in vertebrates playing the role in specifying neuronal fate. The aim of this study has been to determine and characterize the promoter of the human SOX3 gene and to elucidate molecular mechanisms underlying the regulation of its expression. We have identified the transcription start point and carried out the structural and functional analysis of the regulatory region responsible for SOX3 expression in NT2/D1 cell line. Using promoter-reporter constructs we have determined the minimal SOX3 promoter region that confers the basal promoter activity. We have investigated in detail the functional properties of three conserved motifs within the core promoter sequence that bind transcription factors Sp1, USF and NF-Y. By mutational analysis we have shown that all three sites are of functional relevance for constitutive SOX3 expression in NT2/D1 cells. Taken together, data presented in this paper suggest that transcription factors such as Sp1, USF and NF-Y could function as key regulators for the basal activation of the human SOX3 gene.

P1302. Interspecies variance of Alu elements in the galectin-1 gene correlates with splicing isoforms pattern.

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LGALS-1 gene codifies the 14-kDa galactose-binding lectin galectin-1. The protein own multiple cellular functions spanning cell-cell and cell-matrix interaction, cell differentiation, pre-mRNA maturation, and apoptosis induction. Exon skip alternative mRNA of the gene was found in human tissues as well as in normal and tumor cells. Alternative isoform bears premature truncation codon that might influence protein expression.

Previously we suggested that the presence of Alu-S elements in the human gene, one upstream and two others downstream the skipped exon give rise to a stem-loop configuration of the primary transcript affecting mRNA maturation, due to their arrangement in opposite directions (Bruzze et al., ESHG 2004). Here we report comparative analysis of gene expression that further underlines how Alus pairing drives folding of mRNA and may cause exon skipping.

Human gene expression pattern shows normal product, a shorter one lacking the skipped exon and a larger isoform, likely an intermediate mRNA which include introns or part of them.

In monkey the galectin-1 gene possess one Alu-S in the upstream intron, but three in the downstream intron all belonging to the S family and also oriented in inverse direction with respect to the first one. Expression pattern displays normal and exon-missing mRNAs, however it shows two larger transcripts, presumably explained by an augmented potentiality of combinations between the Alus to form secondary structures.

On the contrary, mouse posses only the Alu element in 5'- and none in the 3'-intron, however exon sequence are highly comparable. Noticeably, gene expression completely lost the alternative isoforms.

P1303. Semax increases BDNF expression in ischemized rat brain.

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Neurotrophic factors (e. g. brain-derived neurotrophic factor, BDNF) are known as natural neuroprotectors involved in proliferation, differentiation and survival of neuronal and glial cells. Analysis of neurotrophins mRNA expression is a convenient tool to assess therapeutic efficiency of the anti-stroke drugs. It was revealed that BDNF expression was increased in rat cultured glial cells under

treatment by the neuroprotector Semax (synthetic polypeptide Met-Glu-His-Phe-Pro-Gly-Pro; its N-terminus represents a fragment of adrenocorticotropic hormone). We have analyzed the effect of Semax upon BDNF expression within cerebellum and forebrain cortex of the rats subjected to global brain ischemia. After 15 minutes of irreversible bilateral common carotid artery occlusion the animals were subjected to intraperitoneal injection of Semax or 0,9% NaCl. Animals were decapitated 30 minutes /1 hour / 2 hours after the operation. Sham-operated animals were used as a control. BDNF mRNA expression level was assessed by semi-quantitative PCR. Under experimental conditions, the sham-operated and ischemized rats treated with 0,9% NaCl did not demonstrate the difference between BDNF expression levels within investigated brain regions. BDNF expression within the cerebellum of the ischemized rats also was not influenced by Semax. At the same time, comparing to the ischemized animals treated with 0,9% NaCl, expression level of BDNF within forebrain cortex of the animals treated with Semax was increased. Maximum of the BDNF expression was detected within the forebrain cortex 30 minutes after occlusion. Possibly, therapeutic effect of Semax is mediated by the increased production of BDNF within brain cells.

P1304. Gene Filter: a software tool for high throughput sequence selection and analysis.

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For research organisations with limited bioinformatics support, there are time restraints in the analysis of large numbers of target sequences. *Gene Filter* is a user-friendly java software for high throughput sequence selection and analysis for multiple purposes such as the design of customized oligo microarrays. The current release includes *Gene Finder* and *Sequence Extractor*.

Gene Finder is a search engine that queries NCBI and UCSC databases for gene related information, chromosomal coordinates and strand orientation for a large number of user defined search identifiers such as GenBank, RefSeq, UniGene, GeneSymbol, CytLocus or nucleotide sequences. Search terms are either loaded from plain text files or directly pasted into the program window. Results are displayed in the program window. Several user defined selection features allow additional filtering of search results.

Sequence Extractor is an automated sequence deriving routine that uses the strengths of UCSC Table Browser and NCBI Map viewer to extract multiple sequences and annotations for *Gene Finder* defined genome regions.

Userfriendly batch processing of hundreds of sequences can be performed very efficiently for high throughput sequence analysis. Further program packages for the design of gene expression-, SNP-, CGH-, and methylation microarrays are under development.

P1305. Simple solutions for multiplexed real-time PCR with Promega's new Real-Time PCR System

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Promega Corporation, Madison, WI, United States.

Join Promega for the introduction of our new Real-Time PCR Technology, **Plexor™ System**. Based on the expanded genetic alphabet, the system allows simple design of multiplexed real-time PCR assays for genotyping and quantitative analysis. Data will be presented on new assay designed for RNA or cDNA gene expression analysis, SNP genotyping, mutation detection and detection of bacteria/virus. Understand how the system will simplify your assay development, allow you to develop high performance primers on our website, give you the ability to run high multiplexed quantitation with one chemistry on any real-time PCR instrument and enhance your data analysis with the straightforward system software.

P1306. Impact of genetic polymorphisms on birth weight

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All studies from Poland and the Czech Republic indicate the relationship between ambient air pollution and an increase of DNA

adducts in maternal and cord blood and/or in placentas, as well as the relationship of these biomarkers to the development of newborns. Therefore we studied the impact of genetic polymorphisms on pregnancy outcome as a part of EC CHILDRENGENONETWORK project. DNA was isolated from placenta samples collected in polluted regions (Teplice and Prague) and control region (Prachatic). Polymorphisms of metabolic genotypes (GSTM1, GSTP1, GSTT1, EPHX3, EPHX4, CYP1A1 Ile/Val and CYP1A1-Mspl) were determined in 1014 placenta samples. The consequence of genotypes to birth weight as well as category LBW+prematurity (low birth weight < 2500 g, prematurity < 37 weeks) was determined. Using multiple regression analysis, CYP1A1-Mspl mutation decreased the newborn birth weight of smoking mothers in Teplice (-315 g, $P<0.05$). GSTM1 null genotype significantly increased LBW+prematurity in all three groups (AOR = 1.33, $P<0.05$, especially at Teplice AOR = 1.54, $P=0.01$ and Teplice-nonsmokers AOR = 1.53, $P<0.05$), CYP1A1 Ile/Val polymorphism in smoking mothers in the first trimester (AOR = 2.89, $P<0.05$) and smoking mothers in the first trimester at Teplice (AOR = 2.91, $P<0.05$). Other significant predictors were exposure to carc-PAHs, maternal smoking, passive smoking. Combination of different polymorphisms is further analyzed. Our results indicate that genetic polymorphisms may affect birth weight in newborn children, if combined with air pollution and/or lifestyle (smoking).

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P1307. Analysis of iNOS expression in rats under brain experimental ischemia

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Stroke is the second or third leading cause of death in many countries. Although the underlying mechanisms of this disease are not fully understood, it has been shown that nitric oxide (NO) overproduction and inducible nitric oxide synthase (iNOS) overexpression play important roles in producing injury caused by hemorrhagic shock. To investigate the profile of iNOS expression during the first 24 hours after ischemia we took advantage of the experimental model of the global brain ischemia in rats. Ischemized animals were decapitated 30 minutes / 1 hour / 2 hours / 4 hours / 8 hours / 12 hours / 24 hours after irreversible bilateral common carotid artery occlusion. Forebrain cortex iNOS mRNA expression level was assessed by RT-PCR. iNOS low-level expression was first observed 8 hours after the operation; we did not detect any expression activity of the gene immediately after the operation. Within 12 and 24 hours after the operation the level of iNOS expression was increased insignificantly. Thus we demonstrate that under the experimental conditions used the level of iNOS expression is low throughout first 24 hours after the operation.

P1308. Validation of the novel chemiluminescent microarrays using real-time RT-PCR

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Over the recent years, gene expression profiling has become a promising tool for the risk assessment and therapy prediction of cancer. In contrast to the fluorescent technology used by other platforms, the novel Applied Biosystems 1700 chemiluminescent microarray analyzer uses a chemiluminescent substrate following digoxigenin-labeling and incubation with anti-digoxigenin-alkaline phosphatase antibody. Chemiluminescent detection increases the sensitivity of expression analysis, and the use of 60mer oligonucleotides supports high specificity.

We have tested the precision and accuracy of this novel microarray system. Total RNA was isolated from fresh frozen tumour samples of 7 patients with ductal invasive breast cancer. All samples were analyzed with the Applied Biosystems Human Genome Survey Microarray (HGSM). Technical replicates ($n=5$) showed high reproducibility of the array results. Real-time RT-PCR was used as an independent method to validate the gene expression levels found by microarray analysis. For real-time RT-PCR 71 breast cancer-related genes were selected and

analysis was performed using the gene expression assays on demand (Applied Biosystems). For the comparison of HGSM data and RT-PCR a Pearson-correlation of 0.92 to 0.63 was found for the established cancer genes, e.g. EGFR, erbB2, estrogen receptor α (ESR1), progesterone receptor (PGR), urokinase-type plasminogen activator (PLAU, uPA), and plasminogen activator inhibitor-1 (SERPINE1, PAI). In conclusion, the novel 1700 chemiluminescent microarray analyzer generates highly reliable and accurate data when performing whole genome expression profiling. Therefore, highly sensitive and specific microarrays may become a useful tool for clinical applications.

P1309. Different quantitative traits of gene expression in idiopathic dilated, hypertrophic and restrictive cardiomyopathy

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Background. The ability to quantitate the expression levels of specific genes has always been central to any research into gene function and gene-to-gene interactions. We tested the hypothesis that different gene expression of cytoskeleton, contractile proteins MMP and related TIMP characterise idiopathic dilated, hypertrophic and restrictive cardiomyopathy, demonstrating that gene expression profiling of myocardium may meet clinical parameters and is feasible in the cardiological setting.

Material and Methods. Total RNA was extracted from 70 myocardial samples (both left and right ventricles) obtained from hearts excised at cardiac transplantation (San Matteo hospital). cDNAs were analysed by real time PCR for the following genes: MYH7, MYBPC3, TNNT2, ANGPT2, GAA, TIMP1, TIMP4, MMP1, MMP2, MMP9, MMP10, MMP11, MMP12, MMP14. The between-group differences were analysed using the by Mann-Whitney U test. Age, gender, and hemodynamic differences of the samples did not affect the profile's accuracy in stratified analyses. We also correlated the gene defects with the expression patterns.

Results and Conclusions. We found a substantial over expression pattern of the cytoskeletal/contractile proteins MYH7, MYBPC3, TNNT2 in the groups but with a number of quantitative differences. Moreover, the over expression of ANGPT2, GAA, TIMP2 and TIMP4 profiles the hypertrophic pattern while the over expression of MMP1, MMP2, MMP9, MMP10, MMP11, MMP12 and in particular MMP14 characterises the dilatative pattern ($p<0.01$). We also found that gene profiling is specific to disease stage, and it is unaffected by differences in clinical characteristics. Finally, these studies may prompt further pharmacological investigations in identifying specific pathways for therapeutic targeting.

P1310. Non-enzymatic labeling of nucleic acids using The Universal Linkage System (ULS™) in DNA Microarray 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. In DNA microarray applications the ULS™ system demonstrates one of its key advantages: direct labeling of nucleic acids in their natural form, e.g. genomic DNA, total RNA or its mRNA fraction.

Current labeling methods in microarray experiments rely on enzymatic conversion of the original target into labeled cDNA or aRNA. Apart from being time consuming, costly and error-prone, valuable information is lost due to bias introduced by the enzymes. Using ULS™ to directly label natural targets circumvents this problem and also gives major advantages in studies to e.g. splice variants or microRNAs. Here data will be shown in which the ULS™ arrayCGH fluorescent labeling kit has been evaluated for direct genomic DNA labeling used for BAC array hybridizations and compared with standard enzymatic random-prime labeling.

Since the available amount of total RNA can be limiting, some applications require linear target amplification. ULS™ offers the possibility to enzymatically generate unmodified linear amplified aRNA samples which can be used or labeled as required. Importantly, by avoiding the use of modified nucleotides in the IVT reaction, RNA

samples will be amplified with much higher yields and better size distribution of the amplified product. Data will be presented where the ULS™ arRNA fluorescent labeling kit is compared to a commercial aminoallyl labeling product.

P1311. Segmental duplications of human chromosome 8p23.1: multiple copies of a new gene showing variability in copy number in humans and other primates

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Segmental duplications (SD) are paralogous segments of high sequence identity that account for about 5% of the human genome. Due to the high level of identity, these regions are susceptible to illegitimate recombination. Genomic architecture of cytogenetic band 8p23.1 is of special interest due to the presence of low copy repeats located at their boundaries. This particular organization increases the vulnerability of the region to undergo rearrangements, such as a polymorphic inversion found in about 25% of the general population. In this study, we focus on a multiple copy gene distributed in different clusters along the 8p23.1 low copy repeats. Different polymorphic clusters in copy number of a novel gene with unknown function, have been detected by in silico analysis of the 8p23.1 region. These results have been confirmed by pulsed field gel electrophoresis in several subjects of the general population, as well as in chimpanzee, gorilla and orangutan. However, although it is also present in great apes, the organization and copy number of the different clusters are significantly different between humans and great apes. Further exploration of the region 8p23.1, and specially these gene clusters, is of great interest, due to its possible evolutionary implications. Moreover, gene expansion phenomena has been described as a main evolutionary force driving specific traits of the human lineage. Therefore, a better characterization of the human chromosome 8p23.1 region, taken as an example of gene expansion, should lead to a better understanding of common mechanisms underlying evolution of primates and humans.

P1312. 'arrayCGHbase': a freely available and versatile tool for data mining and visualisation of arrayCGH and SNP chip data

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The availability of the human genome sequence as well as the large number of physically accessible oligonucleotides, cDNA, and BAC clones across the entire genome has triggered and accelerated the use of several platforms for analysis of DNA copy number changes including microarray comparative genomic hybridization (arrayCGH) and SNP chip analysis. One of the challenges inherent to this new technology is the management of large numbers of data points generated in each individual experiment. We have developed arrayCGHbase, a comprehensive analysis platform for arrayCGH experiments. ArrayCGHbase consists of a MIAME (Minimal Information About a Microarray Experiment) supportive MySQL database underlying a data mining web tool, to store, analyze, interpret, compare, and visualize arrayCGH results in a uniform and user-friendly format. Following its flexible design, arrayCGHbase is compatible with all existing and forthcoming arrayCGH platforms. Data can be exported in a multitude of formats, including BED files to map copy number information on the genome using the Ensembl or UCSC genome browser. ArrayCGHbase is web based and platform independent and therefore allows users to access the analysis suite through the internet or a local intranet after installation on a private server. We will present the major features of the tool as well as illustrate the potential for custom and future options. ArrayCGHbase is freely available at <http://medgen.ugent.be/arrayCGHbase/>.

P1313. The Regulation of Spermatogonial Stem Cell-Specific Genes by Piwi2 Protein

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Although spermatogenesis is essential for reproduction, little is known about spermatogonial stem cells. These cells provide the basis for spermatogenesis throughout adult life by undergoing self-renewal and by providing progeny that differentiate into spermatozoa. Members of piwi gene family play essential role in self-renewal of germline stem cells. Piwi2 is one of three mouse homologues of piwi. Piwi2 was found in germ cells of adult testis, suggesting that this gene functions in spermatogonial stem cell self-renewal. In order to find molecular mechanisms underlying stem cell activity, *in vitro* gain of function cell culture model was established. Messenger RNAs isolated from cells expressing Piwi2 and mRNAs isolated from cells without Piwi2 expression were compared using a stem cell array technique. It was shown that Piwi2 modulates expression of stem cell specific genes, including Pdgfrb, Slc2a1, Gja7 and spermatogonial cell surface markers Thy-1 and Itga6. These molecules play essential role in non-germinal stem cells.

P1314. The role of the general transcription factor NF-Y in the regulation of the expression of human Sox3 gene

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Early neurogenesis and neuronal differentiation are precisely controlled by a series of genes. Sox3 is expressed in the brain from the earliest stages of development. It is considered to be one of the earliest markers in vertebrates playing the role in specifying neuronal fate. In order to elucidate molecular mechanisms underlying the regulation of the human SOX3 gene expression, computer prediction software was used to search the matrix database and to identify potential transcription binding sites in the human SOX3 promoter. The number of putative consensus binding sites for known transcription factors was identified in the 5' noncoding region of the human SOX3 gene. The presence of three evolutionary conserved CCAAT boxes, representing the putative binding sites for the general transcription factor NF-Y, suggests the potential importance of this factor in the regulation of the human SOX3 gene expression. EMSA and "supershift" experiments are performed to prove the specificity of the NF-Y binding to all three identified CCAAT control elements.

To examine whether the putative CCAAT box is functional, site directed mutagenesis was done. The ability of the mutant and its wild-type counterpart to drive expression of the cat reporter gene was compared in NT2/D1 cell line. We have shown that mutagenesis of the CCAAT box (-101 bp to -105 bp) reduced the expression of the reporter gene more than 5-fold when compared to the wild-type expression. This result indicates that the CCAAT box motif present in the SOX3 promoter plays a functional role in the transcription of this gene.

P1315. Microrearrangements of human chromosome 15q11-q13 in families with autistic disorder

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Autism is a neurodevelopmental disorder exhibiting complex genetic etiology. Human chromosome 15q11-q13 is a candidate region to autism based on linkage, linkage disequilibrium, cytogenetic abnormalities, and maternal duplications at this locus in patients with autism and autism-related phenotype. Chromosome 15q11-q13 contains several imprinted genes expressed in the brain, including small nucleolar RNA (snoRNA) genes, and is rich in segmental duplications. These sequences promote fairly frequent 15q11-q13 rearrangements resulting in Prader-Willi syndrome, Angelman syndrome, mental retardation and autism spectrum disorder. Among plausible candidate genes for autism in this region is the cluster of the gamma-aminobutyric acid (GABA) receptor genes. In order to detect possible duplcon-mediated submicroscopic rearrangements of 15q11-q13, we have performed the analysis of microsatellite markers in Spanish autistic families, and multiplex families of autistic probands from the Autism Genetic Resource Exchange (AGRE) and control samples. We have found frequent microduplications at 15q11.2 region and at GABA locus

in autistic patients and control samples. Two patients have a paternally inherited microduplication between the genes GABRA5 and GABRG3 whereas other two patients have a maternally inherited duplication within the D15S1021 marker. All the other duplications are the novo microrearrangements. Using quantitative PCR, PFGE, additional microsatellites and single nucleotide polymorphisms in the region we are currently defining the size of these rearrangements, which should help to delineate the relationship between 15q11-q13 alterations and autistic disorder.

P1316. Transcriptional activation via bidirectional RNA polymerase II elongation over a silent transposon promoter

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Transcriptional interference denotes negative *cis*-effects between promoters. Here we show that it can act positively. Bidirectional RNA polymerase II (pol II) elongation over the silent HERV-K18 promoter representing 10^4 similar promoters genomewide activates transcription. In tandem constructs, an upstream promoter activates HERV-K18 transcription, which is abolished by a polyA signal in-between, and restored by polyA signal mutants. TATA-box mutants in the upstream promoter reduce HERV-K18 transcription. Experiments with the same promoters face to face produce similar effects. A small promoter deletion partially restores HERV-K18 transcription, consistent with activation resulting from repressor repulsion by the elongating pol II. Transcriptional elongation over this class of intragenic promoters will generate regulated sense-antisense transcripts, or alternatively initiating transcripts, thus expanding the diversity of mechanisms capable of organismal complexity.

P1317. Genome-wide re-sequencing using Cloned Single-Molecule Arrays™

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Revolutionary new technologies capable of transforming the economics of sequencing are providing unprecedented opportunities for whole genome re-sequencing, as well as genome-wide transcription, epigenomics and genome structure analyses.

Solexa's single-molecule array technology for whole-genome sequencing promises minimal (one-tube) sample preparation, ultra high feature densities and greatly reduced costs, and is ultimately capable of reaching up to five orders of magnitude improved efficiency over current methods. To enable single-molecule methods, we have developed a very robust four-colour DNA sequencing-by-synthesis technology that employs reversible terminators with removable fluorescence. This novel sequencing biochemistry has now been shown to support up to 25 cycles with high fidelity, both in solution and on surfaces. A 25-mer read length represents a key milestone towards accurate human whole genome re-sequencing in that 25 bases permits alignment to the human reference sequence, as shown by a recent study conducted *in silico* by Solexa.

To enable earlier applications of our sequencing platform, we have chosen to leverage our proprietary sequencing biochemistry into cloned single-molecule arrays, which are formed using the surface-amplification method developed by Manteia SA. We have made significant progress in the preparation of such cluster arrays from randomly cut genomic DNA samples and in adapting our single-molecule-compatible surface chemistry to improving the methodology. The clusters arrays have been used with our sequencing-by-synthesis technology, a fluorescence microscope and automated fluidics to sequence small genomes. Our current capabilities in this area will be described, highlighting the number of features analysed, attainable read length and the measured sequencing accuracy.

P1318. Prioritization of positional candidate genes in common complex diseases using gene networks

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Although the majority of common diseases are complex, resulting from many different genes with weak effects, there will only be a limiting number of molecular pathways contributing to disease aetiology. Linkage studies have led to the identification of considerable numbers of susceptibility loci, but lack behind in pinpointing true candidate genes from these regions because they usually span 10s of Mb's. To aid in the identification of causative genes we propose a prioritization method for positional candidate genes, by assuming that the majority of causative genes are functionally closely related.

We used a Bayesian framework to generate a gene network, based upon data from GO, KEGG, BIND, HPRD, several protein-protein interactions experiments, approximately 6,000 microarray experiments and data-mining results from PubMed abstracts. We tested the gene network in over 80 heritable disorders for which at least three disease genes have been identified. Artificial susceptibility loci (~10 Mb) were constructed around each causative gene and the gene network was used to predict per disorder per locus the positional candidate genes. For nearly half of the disorders the analysis of the loci using the gene network performed well, i.e. the true causative genes were identified from the artificial linkage regions.

We have shown that by assuming that causative genes in a specific disorder are usually functionally related, we are capable of predicting the correct positional candidate genes when analyzing susceptibility loci. This method therefore could be valuable for analyzing common disease loci in which the causative genes have not yet been identified.

P1319. Expression profiles of the recovering gut in coeliac disease patients points towards the molecular aetiology

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Coeliac disease (CD) is a common autoimmune disorder affecting the small intestine, and patients show an immunological response to the common food protein gluten, which is present in wheat. They suffer from severe malabsorption due to a dramatic restructuring of the mucosa, but on a gluten-free diet they show clinical improvement accompanied by gradual recovery of the mucosa, as judged by the sequential disappearance of the disease's hallmarks (villus atrophy, crypt hyperplasia, and lymphocytosis).

We performed microarray expression profiling on duodenal biopsy samples from 48 CD patients, across all stages of remission, and 21 control individuals to gain insight into the molecular pathology and the driving genetic factors.

Gene-expression profiling yielded 118 differentially expressed genes ($p < 0.05$), of which 78 were up-regulated and 40 down-regulated. These genes can be grouped into a limited number of different processes. In general, remission is a gradual process leading to down-regulation of immune-related genes and up-regulation of genes involved in re-establishing the normal homeostasis of intestinal mucosa. The picture that emerges is that CD is not only an inflammatory condition that affects the absorption of nutrients, but that underlying this impairment is the lack of terminal differentiation of the enterocytes. Many of the clinical features and complications of CD can be directly linked to molecular pathways highlighted in our study.

Interestingly, some of these genes are located under linkage peaks shared between other autoimmune disorders and CD, suggesting these genes might be implicated in common pathways and considered as potential primary genes in autoimmune disorders.

P1320. Identification of transcription start sites in retinal expressed genes

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The aim of this study is the identification of transcription initiation sites of two retinal expressed genes *RDH12* and *SLC24A2* in order to evaluate the reliability of 5' ends provided by refseq database entries. *RDH12* codes for a photoreceptor specific retinal-dehydrogenase and is implicated in Leber congenital amaurosis. *SLC24A2* codes for a potassium-dependent sodium-calcium exchanger in cone photoreceptor.

Our experimental method employed EST sequence database mining and cross-species comparisons to perform *in silico* assembly and analysis of 5' transcript termini. In addition mRNA from human retina was used for Cap Finder RACE experiments to study and characterize the 5' end of the genes of interest. This led us to define additional transcribed sequences extending to the 5' of the reference sequence entries of the two genes which was confirmed by RT-PCR experiments. Moreover, we also found additional exons that were not present in the databases. The new model for *RDH12* contains three new 5' untranslated exons, one of which occurs in two different splice variants. The new model for *SLC24A2* predicts two sites of transcription initiation as well as two additional exons that are alternatively spliced. Experimental analysis of the transcripts proved to be essential for the ultimate mapping of the initiation site. This information constitutes the basis for further exploration of the promoter and other cis-regulatory sequences at the 5' end of these genes.

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P1321. Interactions of 4F2hc and y⁺LAT1 in forming a cationic amino acid transporter

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4F2hc and y⁺LAT1 form together a transporter for cationic amino acids located mainly in the basolateral membrane of epithelial cells in the small intestine and kidney tubule. Mutations in y⁺LAT1 cause lysinuric protein intolerance (LPI, OMIM #222700), which is due to a defect in the absorption of cationic amino acids in the small intestine and the proximal kidney tubule. 4F2hc, which is a 529 amino acids long glycoprotein, interacts with y⁺LAT1 with its carboxyl terminus, but the precise regions taking part in the interaction are still unknown.

We made four different-sized deletion constructs (262, 278, 321 and 404 amino acids) of 4F2hc to identify the most necessary regions of the carboxyl terminus in the interaction with y⁺LAT1. The significance of the disulfide bridge between 4F2hc and y⁺LAT1 was also examined by mutating the bridge-forming cysteine residues into alanines. In order to study the interaction of the subunits we labelled the C-terminal end of 4F2hc with a DsRed1 tag and y⁺LAT1 with an EGFP tag and studied the trafficking of the two proteins to the plasma membrane in the HEK 293 cells with a confocal microscope. The most important regions of the carboxyl terminus of 4F2hc included amino acids 279-321 and 405-529. The absence of the disulfide bridge did not have any effect on the trafficking of the transporter to the plasma membrane. Currently, we are making a more detailed deletion analysis of the C terminus of 4F2hc in order to locate the most important interaction region.

P1322. Highly conserved noncoding DNA sequences within introns are controlling in cis the expression of GLI3

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Limb defects present an excellent model for the study of signaling pathways in humans. Molecular clues involved in limb patterning are similarly used to direct the development of other parts of the body.

The products of the GLI gene family translate signals of the sonic hedgehog protein (SHH) into specific patterns of gene expression. Their co-ordinated function appears to determine a GLI-code which, in the limb, directs pattern formation in posterior-anterior direction. Factors controlling the localized and timely expression of GLI genes are unknown.

We report the identification and functional analysis of cis-regulatory elements controlling expression of GLI3.

The genomic sequence upstream of exon 1 of human GLI3 is predicted to contain a promoter sequence. By deletion analysis, we identified a 300 bp minimal promoter region with a high capacity for transcriptional activation of a luciferase reporter gene in cell culture. To assay the involvement of trans-active factors, predicted binding sites within this region are modified by mutagenesis.

Comparison of the human, mouse and fugu genomic GLI3 sequences showed regions of very high conservancy residing in intronic regions. Three such segments were tested for their potential to regulate luciferase expression in cell culture. Two segments differing in these properties were further analyzed for their ability to control time and localization of beta-galactosidase reporter gene expression in transgenic mouse embryos.

The detection of sequence elements controlling in cis the expression of GLI3 contributes to the understanding of pattern formation and addresses the question of highly conserved noncoding DNA sequences in vertebrate genomes.

P1323. SOX gene expression analysis by non-radioactive RNA-RNA in situ hybridization

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RNA-RNA in situ hybridization is reliable method for studying tissue and cell specific gene expression, which enables visualization of labeled antisense RNA probe hybridized to specific mRNA. In this study we have employed non-radioactive RNA-RNA in situ hybridization using biotin- or digoxigenin-labeled RNA probes in order to detect SOX gene expression in carcinoma cell lines. By applying this approach we confirmed results obtained by Northern blot analysis, where presence of SOX2 mRNA in NT2/D1 and SOX14 mRNA in HepG2 cells has been shown. Our aim was to set up RNA-RNA in situ hybridization method in in vitro cultured cells in order to further analyze SOX gene expression on various normal and cancer tissues.

P1324. A subset of miRNA genes are associated with CpG-islands

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Micro-RNA (miRNA) genes encode small RNA molecules involved in mRNA translation and degradation by the RNA interference (siRNA) machinery. CpG-islands are CpG-rich regions that are common near transcription start sites, often associated with promoter regions, and some of which may be hypermethylated in cancer. We have found a highly significant proportion of miRNAs (11.5%) embedded within CpG islands. This includes has-mir-212 and has-mir-132, which are located in the CpG-island of HIC1 (hypermethylated in cancer 1). Although CpG-islands are frequently associated with the 5'-region of known genes, 8000 of the 27000 CpG islands in the genome are located in between genes. We found that a subset of the miRNAs are intimately associated with these non-genic CpG-islands as well, with a significant bias towards a location of the CpG-island within a 5 kb upstream region of the miRNAs. Thus, a subset of miRNA genes are associated with CpG-islands, suggesting a potential role of these miRNAs in CpG-island methylation, in development and in carcinogenesis.

P1325. UMD-FBN2: A New Locus Specific DataBase (LSDB) for mutations in the FBN2 gene

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Congenital Contractural Arachnodactyly or Beals-Hecht syndrome is a heritable connective tissue disorder caused by mutations in *FBN2* gene. It is related to Marfan syndrome (MFS) which is due to mutation in another member of the same family, *FBN1*. Main symptoms of CCA are skeletal features as arachnodactyly, dolichostenomelia, pectus deformities and kyphoscoliosis. This disease is associated with a relatively good prognostic because of the rare occurrence of cardiovascular manifestations as aortic dilatation or dissection and mitral valve prolapse, responsible for the most important mortality in MFS. The incidence of CCA is unknown, but it is rarer than MFS which incidence is 1/10, 000. *FBN2* is a 127,6 Mb gene in 5q23-31 encoding fibrillin-2. It shares with *FBN1* an interesting structure of tandemly repeated modules. Because of the high similarity between these two genes, it seems to us interesting to be able to compare their mutations. For this purpose, we have constructed, as for *FBN1* in 1995, a locus specific database with the UMD software. The database currently contains 22 *FBN2* mutations, 21 of which are clustered in exons 24 through 34. They include 1 nonsense and 8 splice site mutations predicted to result in shortened fibrillin-2 molecules. We have annotated the *FBN2* sequence with described Highly Conserved Domain (HCD). These data allows us to list missense mutations as following: 3 mutations are in amino acids implicated in Ca^{2+} binding, 8 mutations modify cysteines implicated in disulfide bond, and 1 mutation is in a conserved amino acid in TGFBP modules.

P1326. Visualization of 1039 chromosomal breakpoints in Mendelian Cytogenetics Network database (MCNdb) associated with mental retardation

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Mental retardation, affecting 2-3% of the population, is an extremely heterogeneous condition where probably hundreds if not thousands of genes might be involved. Mendelian Cytogenetics Network is a collaboration of >300 cytogenetic laboratories that submit disease-associated balanced chromosomal rearrangements (DBCRs) to a central database MCNdb (<http://www.mcnbd.org>). Presently, there are 2852 DBCRs in MCNdb and the most common trait is mental retardation (1039 DBCRs ~36% of DBCRs in MCNdb). We have initiated a systematic analysis of this subgroup in order to identify good candidates for further molecular mapping by using tools available within MCNdb. The tool Genome Link displayed 2237 breakpoints in the UCSC Genome Browser, facilitating the detection of breakpoint clustering. At the genome level, the 5 largest clusters involved 7q11, Xp11, 7p16, 2q31 and 7q22. The tool Trait Compare compared clinical traits within selected clusters in order to detect potential identical syndromes. We applied general network theory to search for co-occurrence of mental retardation with any other trait. Breakpoints involving chromosome 1 were most frequently associated with speech defects, behaviour disorders and hypotonia, whereas breakpoints involving chromosome 2 were most frequently associated with dysmorphic facies, seizures and paresis of ocular muscles. Furthermore, we used the tool Check OMIM to link the breakpoints and associated traits in specific cases to known loci in Online Mendelian Inheritance in Man. Our data supports that virtual analysis of DBCRs associated with MR by the tools available within MCNdb can identify candidates for further mapping studies and clinical re-examination.

P1327. Expression analysis of human INSL3-LGR8 system

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INSL3 is a member of the Insulin-like family of peptide hormones. It's mainly produced by Leydig cells in prepubertal and adult testis. This peptide is involved in transabdominal descent of testes acting through its only G-protein coupled receptor LGR8. A paracrine role is described in preventing male germ cell apoptosis in rat. INSL3 and LGR8 mutations are described in patients affected by cryptorchidism. We analyzed INSL3-LGR8 system expression, both at RNA and protein level, in various tissues and we tested the hypothesis of imprinting in INSL3 studying three frequent polymorphisms.

INSL3 and LGR8 transcripts were contemporaneously found in different

tissues (skeletal muscle, ovary, testis and pituitary gland) thus suggesting new paracrine roles of the peptide-receptor system.

In testis, ovary and lymphocytes, INSL3 presents an additional transcript due to an alternative splicing introducing a 95bp fragment between exons 1 and 2. Probably this new exon comes from an ALU sequence. The additional transcript is not present in other tissues. Both INSL3 alleles are expressed in patients' cDNA, thus excluding imprinting hypothesis.

The putative INSL3 peptide translated from the alternative transcript, and seen by Western Blot, shares the C-term sequence with JAK3 (Janus kinase 3) protein. JAK3 and INSL3 are contiguous genes in 19p13 sharing one exon. Normally this exon is translated in the two proteins with different frames. The alternative INSL3 transcript uses the JAK3 frame. This might suggest a common mechanism involving JAK3 and INSL3 minor form. It might also be just a not useful transcript induced by the ALU insertion.

P1328. Regulation of alternative splicing in the *MID1* gene causing Opitz BBB/G syndrome

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Clinical features of Opitz BBB/G syndrome are confined to defects of the developing ventral midline, whereas the causative gene, *MID1*, is ubiquitously expressed. We have shown previously that the *MID1* protein function is restricted to the developing ventral midline by a large number of alternatively spliced transcripts that function in a negative regulation mode.

Analysing the alternatively transcribed exons in more detail, we have now discovered that two of them are partially derived from ALU repeats. Point mutations have led to splice donors and consequently to novel gene products in the human lineage. Moreover, we have detected 30 additional ALU sequences within the genomic sequence of the *MID1* gene, two of them resembling the exonized ALU's. These sequences are currently tested for potential expression using RT-PCR. However, aberrant expression pattern of alternatively spliced transcripts are a promising pathomechanism in those patients with X-linked Opitz BBB/G syndrome that have no detectable mutations in the open reading frame of the *MID1* gene. Thus, to identify regulatory regions we conducted a thorough analysis of exonic and intronic DNA sequences based on the conservation pattern between six vertebrate species. In addition, we searched the exons for potential binding sites of splicing enhancers. In total, we have identified 40 significant sites on which two prediction methods agree. Eight of these are evolutionarily conserved and thus represent good candidates for functional regulatory sequences. The set comprises binding sites of SRp40, SRp55, ScR35 and SF2/ASF. Mutation analysis and binding tests of the respective sequences are ongoing.

P1329. MAPH as a robust and convenient assay for assessing gene copy number

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DNA copy number variation is an important cause of genetic disease. Multiplex Amplifiable Probe Hybridization (MAPH), a method patented by the University of Nottingham, is a versatile and simple technology for assessing gene copy number. Ease of probe design and manufacture makes MAPH a particularly adaptable technology. Custom probe sets can be rapidly created to target copy number changes not catered for by other tests.

MAPH has proved reliable in research but in order to bridge the gap between its current status as a laboratory test and its projected role in routine clinical diagnosis, a thorough and systematic experimental testing of the assay was needed. A 2-year Wellcome Trust project specifically designed to improve the reproducibility and robustness of MAPH has shown remarkable progress in the precision and reliability of the test. Prior to this development work, measurement errors (expressed as standard deviations) for subtelomeric copy number were generally in the range 10-15%; by adopting an updated, simplified protocol, standard deviations below 7% are consistently achieved on 95% of 100 tests attempted. Other achievements to date include reduction of the amount of DNA required per test, improved probe sets and adaptation to a 96-well plate format. Probe sets have been

validated for loci and genes including subtelomeric regions (ST3F), BRCA1, HNPCC, PMP22 and TBX5.

This project is near completion and has demonstrated MAPH has the potential to work as a diagnostic test and as a complementary technology to confirm results found by other methodologies.

P1330. Large scale SNP genotyping using APEX technology on an *in situ* synthesized microarray

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Arrayed primer extension reaction (APEX) technology was designed as a method for SNP genotyping, mutation detection and DNA resequencing. The capacity for large-scale genome analyses is still critical to many potential applications keeping in mind large number of the good quality SNPs in the databases. For the spotted arrays selection of informative and well-working SNP is still costly and time-consuming process.

We present the different approach that solves the problem by using *in situ* synthesized (5'-3') oligonucleotide arrays on GenomOne platform of LUMA biotech (Germany) for the assay development. We could easily design the set of oligonucleotides and quickly generate as well to test them, and then only informative and well-working SNPs will be selected for the spotting. Spotted arrays are much more economical to use in the large-scale studies compared to Genom platform. In this way we will save the cost of the oligonucleotides (40 EUR per SNP) which will be discarded after 50 to 100 experiments because they are either monomorphic in population under study or do not work in the particular assay (10-20%).

We introduce steps of the final array design as well as first results in designing enzymatic reaction on *in situ* synthesized oligonucleotide microarray. To achieve positive proof for APEX experiments on GenomOne, were designed chip, which contains different test-probes (15-25 nt) coding for the same SNPs and negative control-sequences. As a result of the negative (without template) APEX, detected signals were specific and indicated the higher signal for longer hairpin with higher GC-content.

P1331. CGHPRO - A comprehensive data analysis tool for array CGH

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DNA array based comparative genomic hybridisation (array CGH) is a high resolution screening technique for the genome wide detection of chromosomal imbalances.

In order to cope with the increasing amount of array CGH data we have developed a user-friendly and versatile tool for the normalization, visualization, breakpoint detection and comparative analysis of array CGH results. The program called CGHPRO is a stand-alone JAVA application that guides the user through the whole process of data analysis. The import option for image analysis data covers several data formats. Various graphical representation tools assist in the selection of the appropriate normalization method. Intensity ratios of each clone can be plotted in a size-dependent manner along the chromosome ideograms. The interactive graphical interface offers the chance to explore the characteristics of each clone, such as the involvement of the clones sequence in segmental duplications. Circular Binary Segmentation and unsupervised Hidden Markov Model algorithms facilitate objective detection of chromosomal breakpoints. The storage of all essential data in a back-end database allows the comparative analysis of different cases. The diverse display options not only ease the definition of shortest regions of overlap, but also simplify the identification of patterns of chromosomal aberrations. A special script is dedicated to the recognition of clones that are misaligned or repeatedly show unreliable ratios.

CGHPRO is a comprehensive and easy-to-use data analysis tool for array CGH. Since all of its features are available offline, CGHPRO may be especially suitable in situations where protection of sensitive patient data is an issue.

P1332. Array CGH reveals accumulation of chromosomal breakpoints in regions with high content of low copy repeats in mentally retarded patients

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In a recent publication chromosomal aberrations were reported in 24% of mentally retarded patients investigated. In this array CGH study we analysed a set of 29 patients with mental retardation, who were pre-selected for the presence of abnormal karyotypes by chromosomal HR (High Resolution)-CGH.

We started with a 1Mb resolution for the whole genome (4225 clones; 20 cases) and subsequently supplemented this array with the tiling path for the chromosomes known to be affected (14182 BAC clones; 9 cases).

Array CGH confirmed all aberrations detected by HR-CGH, including a 1.7 Mb deletion on chromosome 17, which was already suggested by HR-CGH.

Although this proved HR-CGH to be highly sensitive, array CGH enabled a more precise localisation of the breakpoints and provided a better understanding of the complexity of chromosomal rearrangements, which was underestimated by HR-CGH

in some cases. Using our array CGH analysing software CGHPRO and a customized script to screen against the segmental duplication database, we examined the distribution of low copy repeats in the regions of chromosomal breakpoints in those nine aberrations that were detected at the tiling path resolution. In 5/9 cases both breakpoints were characterised by an increase of low copy repeats in the breakpoint flanking region, in a further 2/9 cases the regions around one breakpoint showed this feature.

This study represents not only a direct comparison between chromosomal and array CGH techniques, but also demonstrates the potential of array CGH to provide quick insights into the most probable causes of the chromosomal rearrangement.

P1333. Mutational Profiling of Human Disease Genes

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Identification of mutations in human genes to determine the genetic basis of common disorders such as cancer, neurological diseases, autoimmune diseases, and cardiovascular is a challenge. The use of single-nucleotide polymorphisms (SNPs) as markers in complex disease genes, and initiatives devoted to the identification and mapping of SNPs throughout the human genome are ongoing. SNPs or insertions and deletions in populations are detected by a combination of techniques such as sequencing and hybridization. Sequencing is the preferred method to discover and confirm genetic variation. Researchers who have chosen to sequence their gene of interest have to invest considerable amount of time testing the primers they designed. To eliminate the time consuming step of designing, optimizing and validating of PCR primers for human disease genes, Applied Biosystems has developed VariantSEQR™ Resequencing System. Mutation profiling in humans by resequencing has been made simple by this system since it can be easily integrated into any sequencing pipeline. The system takes advantage of the automated capillary electrophoresis platform, as well as reagents and SeqScape® v2.5 software for mutation detection and report generation.

The universal PCR condition and sequencing protocol allows researchers to:

1. Study genes with dense mutation spectrum and polygenic diseases
2. Undertake case-control studies.

Variations in sequences known or suspected to exist in the human genome can be detected easily and cost effectively. We describe a new tool for mutation and SNP discovery that is simple and easy to adopt, but most importantly employs a proven technique such as sequencing.

P1334. Detailed 5kb density Haplotype Map of Chromosome 2 and 4p

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Whole-genome association studies of common complex traits require genotyping of a large number of genetic markers, which makes such an approach expensive and time- and labour-consuming. Many studies have shown highly significant levels of linkage disequilibrium and strong association between neighbouring single nucleotide polymorphisms (SNPs). These blocks of SNPs create haplotype blocks in the human genome. Generating the map of such haplotype blocks will decrease the number of SNPs needed for genotyping and, therefore, greatly facilitate the mapping and identification of disease-causing genes. The HapMap project is collaboration between 8 groups located in China, Japan, UK, USA and Canada. Our group focuses on creating the haplotype map of chromosomes 2 and 4p. These regions account for 10% of the genome. In the Phase I of the project, we have genotyped about 100 000 SNPs to complete a map with a density of 1 SNP for every 5kb in four populations: CEPH samples from Utah, Yoruba from Ibadan, Nigeria, Han Chinese from Beijing and Japanese from Tokyo. All data are available to the public at <http://www.hapmap.org/>. To manage the genotyping project from sample management to optimal SNP selection, data extraction and analysis our group has developed a web-based application, Nanuq. An algorithm has been implemented to select the best set of tag SNPs from the HapMap data for fine mapping and candidate gene genotyping approaches. Phase II of the project will concentrate on better defining haplotype blocks by creating higher density map of one SNP per 1kb.

P1335. Genomic organisation and expression analysis of the murine basonuclin 2 gene

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We describe molecular characterization and expression pattern of the mouse basonuclin 2 gene, a member of the basonuclin zinc-finger family. The gene has been located in mouse chromosome 4, region C4 and we have found that it contains at least 8 exons spanning approximately 405 kb of genomic DNA. The longest transcript of the mouse basonuclin 2 gene encodes a putative 1106 aa protein with six C2H2 zinc finger domains arranged as three adjacent pairs. The basonuclin 2 protein has also other functional domains, such as 3 nuclear localization signals and a serine stripe.

We have detected mouse basonuclin 2 mRNA expression in 3T6 and NIH/3T3 cells using Northern blot hybridization and RT-PCR. Whole mount *in situ* hybridization revealed expression in 10.5 and 11.5 day mouse embryos, subsequent section of whole mount embryos demonstrated specific expression in ventral dermomyotome and branchial arch region. Mouse MTC panel confirmed expression in 11.5 day embryos and also in 7, 15 and 17 day mouse embryos. In adult mouse we have detected expression in a variety of tissues at different levels.

P1336. Human Whole Genome CGH Arrays Can Detect Copy Number Changes in a Variety of Human Samples

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DNA copy number changes have been implicated in causing human inherited disorders and cancer. Array comparative genomic hybridization (aCGH) allows whole genome coverage and ease of use compared to BAC arrays. Agilent's CGH arrays offer high reproducibility and sensitivity due to major technological improvements in the array slide surface, production robotics, labeling chemistry, and software for quantification and data analysis. The introduction of optimized reagents and protocols for target preparation, hybridization and labeling as well as software for data extraction and analysis has made the detection of changes in copy number such as duplications and gross deletions highly reproducible and extremely sensitive. The sum of these improvements now permits the high throughput analysis of various human DNA samples. Here we describe and demonstrate the

utility of Agilent's whole genome CGH system using cell lines, complex tumor samples, buccal swabs, and whole blood. Comparison with known BAC array data was noted where possible. The whole solution of genome-wide coverage on 44K arrays and optimized reagents and protocols has implications for future diagnostic and prognostic use for specific inherited diseases and cancer.

P1337. Molecular characterization of human Hubert gene located on chromosome 5p13.2

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We have found a novel human gene disseminated over 105 kb genomic DNA in chromosomal region 5p13.2. The gene has 40 exons and it encodes a putative protein Hubert (Human Uncharacterised But Eventually Reasonable Transcript) of 2325 amino acids. Transcription of the Hubert gene occurs in the direction from centromere to telomere. There are three alternative polyadenylation signals (AAUAAA) downstream the stop codon in the sequence.

To investigate the genomic organization of mouse and rat Hubert genes, we performed searches in mouse and rat genome databases. The localization on mouse chromosome 15A2 and rat chromosome region 2q16 is in conserved synteny with the localization of human Hubert on chromosome 5p13.2.

The novel gene is located between genes nucleoprotein (NUP) 155 and NIPBL, the latter being mutated in individuals with Cornelia de Lange syndrome (CdLS).

RNA *in situ* studies were performed on mouse embryos at E9.5, E10.5 and E11.5. Our study demonstrated that Hubert is expressed in E10.5 and E11.5 mouse embryos, but not in E9.5. Expression was detected in cephalic mesenchyme tissue only.

Analysis of gene expression profiles across different tissues was performed using RT-PCR and Northern Blot.

We have not found any conserved domains or motifs in Hubert sequence on protein level using *in silico* analysis. From the experiments and computer analysis performed so far we are not able to predict the function of this novel gene and its protein product. Further analysis is necessary to elucidate the function of Hubert.

P1338. The EMBL-Bank database, and related EBI resources

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The EMBL-Bank database is maintained at the European Bioinformatics Institute (EBI), and stores nucleotide sequence data and associated annotation. EMBL-Bank is part of the International Sequence Database Collaboration (INSD), sharing data with both Genbank and DDBJ. Nucleotide sequence data can be submitted to EMBL-Bank via a dedicated web-based submission system, Webin, and data can be retrieved via FTP, or using SRS, an advanced search system allowing complex cross-database queries.

In addition to the EMBL-Bank primary dataset, several related databases and services are available, including the Third Party Annotation dataset, the EMBL-Align database, the completed genomes webserver and Genome Reviews. The Third Party Annotation dataset allows researchers to add annotation and/or sequence data to nucleotide sequences where they were not the original submitter (something which is not permitted within the EMBL-Bank primary dataset). Submissions must be published in a peer-reviewed journal, and must include new experimental evidence. The EMBL-Align database provides a public repository for both protein and nucleic acid sequence alignment data, storing alignments in a format which is both human and computer readable. The complete genomes server gives access to hundreds of completed genome sequences, all of which are linked to either the original EMBL-Bank entries or Ensembl, while Genome Reviews provides complete genomes with additional manually annotated data derived from the UniProt Knowledgebase, InterPro and the GOA project, providing enhanced annotation of coding sequence features.

P1339. Expression profiling of peripheral blood cells in patients with different subtypes of spinocerebellar ataxias

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Spinocerebellar ataxias (SCAs) are dominant, late onset hereditary disorders characterized by a progressive ataxia that is variably associated with other neurological symptoms. The clinical hallmarks result from a progressive degenerative process that mostly affects the cerebellum, brainstem and spinal cord. To date at least 25 different loci are associated with SCAs and related diseases. A large number of SCAs are caused by expanded CAG repeats within protein coding regions leading to polyglutamine tracts.

Early detection of these disorders would enable both more effective diagnosis and treatment as well as a better understanding of pathogenesis and pathophysiological processes. Therefore, we need easily accessible markers, which should i) differentiate between patients with different SCA types and ii) be detectable already in a preclinical state of disease progression.

To achieve this goal, we collected peripheral blood (PaxGene) of patients with six different SCA types as well as controls for expression profiling. Here we report the results for SCA1, 2, 3 and 6. First, we established a protocol to reduce the globin messages with magnetic beads. After hybridization of the U133 plus 2.0 Affymetrix array, expression profiles for both controls and SCA patients were analysed. Differentially expressed genes were determined that can distinguish between control samples and SCA patients as well as a set of genes which separates SCA3 from SCA6 patients. Microarray results were validated using real-competitive RT-PCR.

P1340. Mirror Image DNA (L-DNA) For Improved Studies on Zip-Coded Universal Microarrays

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Universal zip-codes are a means to combine reactions that occur in solution with the ability to separate molecules on solid support such as DNA-microarrays. To this end, the reactive partners - oligonucleotide primers in our case - are attached each to a unique DNA tag-sequence, whose complement (zip-oligomer) is presented on the surface of the array. Due to their specific tag-sequences, the primers bind to distinct microarray positions and can thus be analysed independently. One difficulty is the selection of the tag sequences. The more complex the mixture of reactive partners gets the more unique sequences are required. In addition, cross-reactivity to the DNA- or RNA-analyte has to be avoided. We present here a zip-code universal array platform based on mirror image DNA (L-DNA). The advantage of the L-DNA derivative used here is the fact that there is no interaction between the L-form sequences and natural (D-form) nucleic acids. In consequence, cross-hybridisation is eliminated, while nevertheless strong and sequence-specific binding occurs between tag and complementary zip-probe. Synthesis of hybrid molecules of normal and L-form DNA is done using chemical standard procedures.

We show here the results of two basic applications of L-DNA universal microarrays. In an epidemiologically motivated project, we analyse candidate single nucleotide polymorphisms (SNPs) that are likely to be disease-relevant by incorporation of labelled dideoxy-nucleotides. For transcriptional profiling of pathogens, gene-specific primers with the zip-code complement attached to their 5'-end are used for the production of zip-tagged, labelled cDNA, following a two-dye competitive hybridisation approach.

P1341. The role of viruses in reproduction and aneuploidy

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Each chromosome contains two centrioles which adhere to each other through the cell cycle and normally separate only once during the G1-to-S cell cycle transition, resulting in centrosome duplication. Centrosome duplication is regulated by many intracellular events that are essential in maintaining genomic stability. Abnormal centrosome

duplication is tightly linked to aneuploidy. Spontaneous abortions appear with the incidence of 12-15% in population. In spontaneous abortions there are 25-60% of chromosome abnormalities, and in most cases there are triploidies, tetraploidies and polyploidies. The genes associated with cell cycle abnormalities are p53, Brca1, Brca2, Gadd45, human papillomavirus type E6 and E7 also leads to mitotic defects. The theory of "two hits" for one unstable cell cycle resulting with aneuploidy is still in bases of these events. From the couples in the genetic counselling process with normal karyotype and aneuploidy in aborted material analysed by flow cytometry, we found CMV and EBV reactivation or new infection in both parents before and/or during pregnancy. We analysed those with aneuploidy in aborted material and significant serologic findings in both parents. A retrospective analysis was done over 500 couples with one or more spontaneous abortion and 296 paraffin embedded samples were found. 41 placentas were analysed by flow: 27 (66%) diploid and 14 (34%) aneuploid. From 290, 88 (30%) had IgM or/and high IgG for EBV and 12 (4%) for CMV. HPV positive diploid paraffin embedded sample from one couple with three consecutive spontaneous abortions was found.

P1342. First Cuban Predictive Testing Experience For Spinocerebellar Ataxia Type 2: A Psychological Follow-Up One Year Later.

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Objectives. The psychological impact evaluation after the predictive experience on descendants at risk of Spinocerebellar Ataxia Type 2, as well as the description of the social and demographic profile of the participants in the Cuban program. **Methods.** A descriptive investigation of series of cases was carried out with 150 individuals at risk who received molecular diagnoses during the predictive protocol started April 2001 and carried out until May 2002. **Results** The average age of the participants was 39 years old, 65,3% of them were women. 83,6% of the participants were married or lived with a partner and 78,3% of them had at least one child. The anxiety and depression levels decrease significantly with time, in direct relation with negative diagnoses in the case of anxiety. **Conclusions.** The psychological well-being indicators improve significantly a year after the predictive experience, which psychosocial impact is favorable. At the same time, the participants assessment on their own experience is satisfactory.

P1343. Investigations of pharmacogenetic features are an important part of predictive genetic counselling

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In memoriam Professor Eugene I. Schwartz

Information on individual pharmacogenetic features (IPGF) as a part of the genetic predictive counseling is the essential basis for prevention of various complications induced with medicines.. The aim of this study is to quantify the rate of four IPGF among St. Petersburg population: butyrylcholinesterase (CHE, EC3.1.1.8, loci CHE1, CHE2), arylamine N-acetyltransferase (AT, EC2.3.1.5), angiotensin-1-converting enzyme (ACE, EC3.4.15.1). **Materials and Methods.** Random blood samples (1350) were analyzed using routine agar gel screening test for CHE1S and CHE1D, spectrophotometric methods for identification of CHE1U, CHE1D, CHE1F, CHE1S; electrophoretic separation in 7,5%PAAG for identification CHE2(5+), Sulfamethazine acetylating method for acetyltransferase phenotype (AT); insertion(I)/deletion(D) polymorphism of the 16th intron of the human Ace gene was determined by PCR with DNA extracted from white blood cells and the product of amplification was analyzed with 8% PAAG electrophoresis and ethidium bromide visualization. **Results.** The frequencies of four PGICH are the following: CHE1U - 0.980, CHE1D - 0.012, CHE1F - 0.008, CHE2(5-) - 0.961 and CHE2(5+) - 0.039; AT rapid allele - 0.272, AT slow allele - 0.728; AceD allele - 0.491 AceI allele - 0.453. Groups of potential risk have been determined and genetic individual information have been registered using special certificate. Hitherto, unfortunately, individual pharmacogenetic information is unclaimed by most our physicians and medicinal management is done with ignorance of IPGF.

P1344. PKU neonatal screening in Leningrad province*I. A. Ivanov^{1,2}, T. S. Sova^{1,2}, L. A. Ryamo^{1,2}, M. O. Mkheidze¹;**¹Medical Academy for postgraduate education, St.Petersburg, Russian Federation, ²District Children Hospital, St. Petersburg, Russian Federation.**In memoriam Dr. Sophia P. Maximova*

PKU neonatal screening has been put into practice by medical genetics service to have been stationed at District Children Hospital. Every year cohort of newborns includes about 10-12 000 persons. Now 98-99% of the infants are examined through PKU neonatal screening with blood dried on filter paper. The incidence of PKU is estimated at 1:6600 - 1 : 12262 live births in Leningrad province. Dietary management with modern Russian and import products like "Tetraphen", "Phenyl-Free" etc. is neonatally started. The goal for management of the infant with PKU is to achieve and maintain blood PHE concentrations between 2-6 mg/dL. All children with PKU (27) have long-term dietary management and PHE level monitoring. Families with PKU probands have a chance to look through a new brochure "Phenylketonuria" written by M. Mkheidze (PhD, MD). DNA analysis and prenatal diagnosis are available with support of Laboratory of prenatal diagnosis (Chief of Laboratory - academician, professor V. S. Baranov) of Institute of Obstetrics and Gynecology RAMS. 10 children of our cohort with PKU have molecular diagnosis.

P1345. Disease knowledge, reproductive attitudes, and compliance to treatment among cystic fibrosis families in Brittany (France).*M. De Braekeleer¹, G. Rault²;**¹Faculté de Médecine & CHU Morvan, Brest, France, ²Centre de Perhardy, Roscoff, France.*

Cystic fibrosis (CF) has an incidence of 1 in 2636 live births and a carrier rate of 1 in 26 inhabitants in Brittany. A questionnaire was distributed to the 506 patients (children, adolescents and adults) and/or their parents followed by the CF centers in Brittany and Loire-Atlantique. The return rate was 40.9%. The knowledge of clinical signs was rather good among patients and their parents (50-68.7%), but that of the genetic transmission was much better (81.4-93.7%). The knowledge of the recurrence risk by the parents resulted in deciding against further progeny or in reducing the number of children. 95.1% of the parents were in favor of prenatal diagnosis, 41.2% having used it. 76.2% would interrupt the pregnancy should prenatal diagnosis revealed that their fetus had CF. All 123 respondents thought that genetic counseling was useful but only 87.1% knew of its availability.

Some 97% of the 207 respondents agreed that the prescribed treatment was very important to maintain their health condition or that of their child. However, 41.1% thought that they or their child took too many drugs. Adolescents and adults were more likely than parents to regularly forget some part of the whole treatment. Compliance to physiotherapy declined among the three categories (from 95.2% to 72.2%). The quality of the relationship between the patient and the health professionals can have a major impact in attaining a better knowledge and a higher compliance to treatment.

Supported by grants from the "Fondation du Centre Hélio-Marin" in Roscoff and the "PHRC"

P1346. GENETICS MADE EASY: free divulgative web about human genetics*M. T. Solé-Pujol¹, J. M. Carrera-Macia², J. M. Cantú-Garza³, F. Solé-Ristol⁴, J. Antich-Femenias¹;**¹Centro Genética Médica, Barcelona, Spain, ²Instituto Universitario Dexeus, Barcelona, Spain, ³Instituto Mexicano del Seguro Social, Guadalajara, Mexico, ⁴Hospital del Mar, Barcelona, Spain.*

Genetics Made Easy, <http://www.geneticsmadeeasy.com> is a non-profit informative web on human genetics that has been written with the aim of bringing the scientific community closer to the general population. The web is a very useful teaching tool to clinicians and other healthcare professionals, in order to complement personal consultations as you will see through the index web, regardless of their area of expertise, as genetically inherited disorders are known across all medical specialties.

The web contains all the information that any couple may need to know before embarking in parenthood, regardless of whether their children were normal or born with any hereditary malformation or disease.

Following a very easy index. Bartolo our owl teacher introduce us from the origin of life to the last new technologies by means of very clear and simple language aided by useful static and flash animated pictures.

The index web is:

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

At this moment <http://www.geneticsmadeeasy.com> is currently available on the Net in English and Spanish. Chinesse during 2005.

P1347. Genetic screening for hereditary haemochromatosis in secondary schools: attitudes of the school community in Victoria, Australia*M. Aitken^{1,2}, S. A. Metcalfe^{1,2}, M. B. Delatycki^{1,3}, K. J. Allen^{1,4}, A. A. Gason^{1,2};**¹Murdoch Childrens Research Institute, Parkville, Australia, ²University of Melbourne, Department of Paediatrics, Parkville, Australia, ³Bruce Lefroy Centre for Genetic Health Research, Parkville, Australia, ⁴Department of Gastroenterology and Nutrition, Royal Children's Hospital, Parkville, Australia.*

Community genetic screening programs can be offered in a variety of settings. Genetic screening for hereditary haemochromatosis has been the subject of significant debate in the literature. Nevertheless, it is the best example of a preventable disease for which genetic predisposition testing is available. Secondary schools are a possible site to offer genetic susceptibility screening, as carrier testing for Tay Sachs disease and cystic fibrosis are currently successfully offered in this setting. Screening within the school system, as an alternative to the current workplace setting, could vastly increase the number of individuals exposed to both the education and the opportunity to be tested. Therefore, we aimed to determine the attitudes of the secondary school community towards a haemochromatosis genetic susceptibility screening program for students without offering testing. Students aged 16-17 completed a questionnaire both before and after an educational session during school time. Parents and teachers received written educational material and returned a questionnaire via mail. Results from the questionnaire study revealed a positive attitude toward genetic testing in schools and a high knowledge level for all participants. In conclusion, comprehensive education is essential to inform students, parents and teachers of all relevant information. The results could inform future implementation and policy development through consideration of community attitudes.

P1348. Genetic Tests under Scrutiny. Development of Criteria Integrated in a Questionnaire in an Interdisciplinary Austrian Working Group*S. Jonas¹, S. Schneider-Voss², G. Endel³, E. Leitgeb⁴, S. Näglein³, D. Vybiral⁵, K. Wimmer⁶;**¹Institute of Technology Assessment, Austrian Academy of Sciences, Vienna, Austria, ²dialog gentechnik, Vienna, Austria, ³Association of Social Insurance Institutions, Vienna, Austria, ⁴Patients Representatives, Vienna, Austria, ⁵Federal Ministry of Health and Women, Department of Biotechnology & Genetic Engineering, Vienna, Austria, ⁶Institute of Medical Biology, Medical University of Vienna, Vienna, Austria.*

In the coming years the number of DNA tests that predict the risk of the development of disorders with genetic influence will increase. Improved technology will make DNA testing more accessible. According to

consumer protection and socio-economic implications there is a need for regulating the approval procedure and cost transfer of those tests. In order to assess the potential benefits and risks an interdisciplinary working group conceptualised a questionnaire regarding evaluation criteria for (predictive) genetic tests.

Under the aspect "*Think genetically, act locally!*" 39 questions based on the established ACCE* criteria were adapted for the situation in Austria. The questionnaire consists of various questions concerning the diagnosis of the disease, test parameter settings, the analytical and clinical validity, as well as clinical utility, and legal and social implications. The preceding preamble gives a short introduction of the detailed work of the participants, an overview of the actual situation of genetic tests and the legal situation regarding the approval of genetic tests in Austria.

The replies of the applicants to these developed questions may help decision makers in health policy to form an opinion about the acceptance or refusal of genetic tests and should help to make the application and decision process more transparent. Also the way (discussion in an interdisciplinary working group) that was used to deal with these circumstances (approval of genetic tests) could be a prototype of how to handle the discussion between different interest groups.

*Analytic and clinical validity, clinical utility, ethical, legal and social implications

P1349. External Quality Assessment in classical cytogenetics: the Italian experience

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Genetic testing services in the EU have substantially increased their activity in the past few years.

In 2002 Italian laboratories performing cytogenetic tests were 158, with an increase of 12% from 2000 (1); cytogenetic analyses performed in 2002 increased of 34% from 2000(1).

The Italian External Quality Assessment (IEQA) in classical cytogenetics is an activity financially supported by the Italian Ministry of health and coordinated by the Istituto Superiore di Sanità, (ISS).

Cytogenetic Public Laboratories have been enrolled upon voluntary participation, covering all Italian regions(2). Four trials have been performed until now and the number of participating laboratories has been increasing (35 in 2001, 46 in 2002, 49 in 2003, 2004-trial in progress). The EQA covers both prenatal and postnatal diagnosis, including cancer cytogenetics.

Laboratories send to ISS Jpeg images and the correspondent written report. A panel of experts evaluate the analytical and the interpretative performances.

Overall images had a good level of quality, while reports were not homogenous and often missing important information. A standard report format, in constitutional cytogenetics, has been proposed by consensus by the ISS and national experts. An overview of all results will be illustrated and effects of the IEQA will be discussed.

1. Dallapiccola et al., Analysis 2/3. 2004: 301-304-available at <http://sigu.univr.it>

2. Taruscio D. et al., Clin Chem Lab Med 2004; 42: 915-21.

This work is funded by the Project "Test genetici: dalla ricerca alla clinica", Italian Ministry of Health (2003-2005)

P1350. Genetic analysis for cystic fibrosis: does it suit me? A blueprint for an interactive educational software

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In the latest years there has been a huge hike in the number of Cystic Fibrosis (CF) carrier tests. Ideally, any genetic test should be linked to an individual genetic counselling session. However, as the number of genetic tests available for clinical use continues to rise, the need for alternative educational and counselling methods is becoming more and more pressing, both for CF and for other inherited diseases. Booklets and videotapes have been used with some success, but they lack interactivity, and often do not include sufficient information to meet the requirements of informed consent. A computer-based program, although not a replacement for genetic counselling, may be

an asset. This kind of tool offers several advantages, like interactivity, adaptiveness, privacy, assessment, and vividness. At present, the plot of the program has been created, and it will be presented. It provides information about CF, genetic risk and genetic testing, and encourages participant interaction by asking users to respond to questions, choose among various options within the program, and access information in the sequence and depth that they desire. Potential users of the CF carrier test should, after using the program, be able to consciously choose whether they want to take the test or not. Steps involving graphic design and software engineering are in progress. Ultimately, a randomized trial comparing education by the computer with education by trained genetic counselors will be performed.

P1351. Cancers, co-morbidities and ageing in people with Down syndrome

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The prevalence of malignancies in people with Down syndrome is controversial, in part because of their major increase in life expectancy over the last 50 years. In a comprehensive study of intellectual disability in Western Australia, linked health databases were used to determine the incidence of specific cancers in 1,298 individuals with Down syndrome (725 males and 573 females) during the years 1982 to 2001. No significant difference was observed between the 21 cancers diagnosed in the Down syndrome cohort and the 19 cases expected in the general population (SIR = 1.10; 95% CI, 0.68-1.68). As in other populations, there was a highly significant increase in childhood leukaemias (n = 12) among Down syndrome patients (SIR = 61.61; 95% CI, 31.84-107.62). Eight other malignancies were diagnosed: cancers of the gastrointestinal system, malignant melanoma, malignant brain tumour, testicular teratoma, cancer of the conjunctiva, and Waldenstrom's macroglobinaemia. The median life expectancy of people with Down syndrome in Western Australia now exceeds 58 years, and many of those diagnosed with cancer had additional health problems, including diabetes, hypothyroidism, epilepsy and musculoskeletal disorders. It has been suggested that the cellular microenvironment in Down syndrome can protect against the development of certain solid tumours, such as breast cancer. However, with their continuing marked increase in life expectancy, it seems probable that in future years people with Down syndrome will exhibit a higher incidence of adult-onset cancers and non-malignant disorders associated with advanced age. This poses serious problems both for Genetic Counselling and public education programmes.

P1352. Education of Medical Genetics in Czech Republic

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Historically the first three medical genetics lectures appeared as a part of the curriculum in Paediatrics at the former Faculty of Paediatrics (now 2nd School of Medicine) in Prague in 1967.

At present there are seven medical schools in the Czech Republic, teaching medical genetics to a variable extent. Most schools teach medical genetics on two levels: First, in the theoretical part of the curriculum (1st and 2nd year), medical genetics is integrated in "Biology". Practicals are implemented to train students in methods like pedigree analysis and risk assessment, methods of molecular genetics and cytogenetics and interpretation of their results, etc. Second, some medical schools implemented obligatory subject "Clinical Genetics" into the clinical part of the curriculum, where students solve practical problems, and learn about the importance of genetics in medicine. In addition, students are also offered optional courses like "Advances in Molecular Genetics", "Genetic Counselling and Clinical Cytogenetics", "Reproduction Medicine and Reproduction

Genetics", etc.

The 3rd Medical School in Prague has a different, problem-oriented curriculum. Medical genetics is taught in the first cycle of integrated study (1st and 2nd year) within "Biology of the Cell" and "Genetics", and in the preclinical part (3rd year) within "Theoretical Foundations of Clinical Medicine".

We have a 3-year postgraduate training programme in clinical genetics for MDs specialized in paediatrics, internal medicine, obstetrics etc. Similar programmes exist for non-medical graduates working in cytogenetic and molecular genetic laboratories.

Medical geneticists are also involved in the education of the public, and in various self-help groups.

P1353. Written informed consent in predictive cancer genetic testing: challenges and pitfalls in clinical practice

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Obtaining informed consent is a legal and ethical requirement for genetic testing for late-onset diseases, including hereditary cancers. However, laws and professional norms concerning written informed consent in clinical practice are often lacking. To explore written informed consent procedures in predictive genetic testing for cancer, semi-structured interviews were conducted with 11 genetic counselors from Toronto, Ottawa, Vancouver and the province of Quebec.

Whereas the process of obtaining informed consent in cancer genetic counseling was homogeneous, variability prevailed in the type of informed consent forms used: the genetic center's own form, a third party's form (from a hospital lab or a commercial lab), a research form, or no form at all. The use of third party forms complicates the issue of the responsibility of clinical geneticists versus that of DNA-labs.

Each participant described the goal of obtaining written informed consent in ethical and/or care related patient-centered terms, stressing that the form cannot replace the informed consent process. Most counselors were reluctant to consider written informed consent as offering legal protection to genetics professionals. Overall, the advantages of written informed consent were perceived as outweighing its disadvantages. Potential for improvement was mentioned, such as more room for patients' wishes regarding re-contact and disclosure to relatives and/or health care professionals. The need for simple language and short forms was emphasized.

Our results show that a more consistent approach is needed for obtaining written informed consent. To meet this requirement, the goals of written informed consent and the ensuing responsibilities involved require clarification.

P1354. Opinions about predictive testing for hereditary breast cancer and Huntington's disease in several groups in Flanders.

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Sixty percent of eligible general practitioners (GPs), 37% of nurses/midwives, 34% of scientists/technicians in human genetics, 44% of members of patient support groups for breast cancer (BC-group) and 78% of members of the Flemish Huntington association (HD-group) expressed their opinions regarding predictive testing for hereditary breast cancer (HBC) and/or Huntington's disease (HD) (1) in an adult, (2) in a 5-year old child at the parents' request and (3) in a 16-year old adolescent at his/her own request. In total, 777 respondents completed a questionnaire.

In each group of respondents, the acceptability ratings for predictive testing for HBC and HD in an adult and in an adolescent are predominantly favourable. Predictive testing for HBC in an adult is rated as more acceptable than testing for HD, mainly because prevention and treatment exist for HBC. In their spontaneous explanations of their ratings, prophylactic mastectomy is mentioned in a negative way by almost half of the GPs, nurses/midwives and the BC-group and by one quarter of the scientists/technicians. Favourable ratings for testing an adolescent were mostly explained by respect for personal autonomy.

Everyone in the HD-group gave a negative rating for predictive testing in a child at the parents' request, whereas negative ratings were observed for 45%-66% of the other groups. Respondents' negative opinions about testing a child at the parents' request were motivated

by the child's right not to know, as well as by psychological and medical arguments.

Implications for genetic education in these groups will be discussed.

P1355. Genetic and phenotypic heterogeneity in Primary Hyperoxaluria type I in Northern Israel

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Primary hyperoxaluria type I (PH1) is a rare autosomal recessive inborn error of metabolism presenting clinical and genetic heterogeneity. PH1 is highly prevalent in Northern Israel with approximately 2-3 new cases each year.

We present 40 patients from 13 Muslim and Druze families. The diagnosis was established based on the clinical presentation, biochemical profile and molecular genetic analyses. Eight different mutations were identified including 6 novel mutations. Patients from eleven families were homozygous for the causative mutation while patients from 2 families were found to be compound heterozygotes. Inter and intra familial phenotypic differences related to age of onset and severity of disease, were observed among patients who shared an identical genotype.

Molecular diagnosis of PH1 was established in 4 patients avoiding liver biopsy, and 1 prenatal diagnosis performed via CVS.

Population screening for the PH1 local causative mutation was performed in two villages. In the first, 190 individuals were tested, and 6 carriers identified, resulting a carrier frequency of 1/31. In the second, 300 individuals were tested, 23 carriers were identified to result a carrier frequency of 1/13. Two couples at risk were identified and prenatal diagnosis (PND) offered.

The high prevalence of PH1 in our region is probably the result of consanguineous marriages. Molecular analysis enables accurate diagnosis and comprehensive genetic counseling for families of affected individuals and couples at risk regarding the options of pre implantation and PND. If PND is undesired pre-emptive liver transplantation is performed preventing end-stage renal disease and improving quality of life.

P1356. Who is developing UK human genetics policy?

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Science knowledge and development of technologies in genetics are changing rapidly which means that policy and legislation concerning human genetics need to be up to date and responsive.

In the UK, there is a wide range of both government bodies and professional organisations who contribute to forming guidelines and policy recommendations which may subsequently lead to legislation on human genetics.

Knowledge about the policy makers and understanding the policy processes involved will help different groups in the UK to access, participate in and become a part of the policy making community. Such groups include those representing families affected by genetic conditions and those involved in genetics research and clinical services. This knowledge is also of interest and relevance to groups outside the UK.

This presentation will describe the main government bodies and professional organisations, their relationships and their roles within the policy making process of human genetics in the UK.

P1357. The Iranian Human Mutation Gene Bank website

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The website of the Iranian Human Mutation Gene Bank represents a comprehensive source of information on DNA samples collected in this bank during the last 6 years. These include DNA of individuals with genetic disorders displaying Mendelian mode of inheritance studied in

Iran. A group of samples have been assigned to common, rare or novel mutations and some others belong to patients with clinical profiles associated with particular genetic diseases but unidentified mutation. The new version of the software presents different grouping of genetic disorders including Hemoglobinopathies, Neuromuscular disorders, Mental Retardations and Hearing loss. Apart from the personal data, which is strictly kept confidential, clinical profile for each individual and genetic data, including pedigree for each family is presented in this database. In order to facilitate collaboration with other scientists in the world with the same interests, we also display the information regarding our experimental projects at this center on some of these genetic disorders. This DNA bank offers a free of charge sample resource from a large heterogeneous population to all the scientists in the world, who are working on various aspects of genetic disorders from prenatal diagnosis to gene structure and function. No commercial benefit is involved in establishment of this DNA bank. Please visit our under construction website on <http://www.IHMGB.com> (Its link is also available in HUGO official website).

P1358. Solving genetic counselling dead end in two Duchenne muscular dystrophy families with the help of three novel microsatellite markers.

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Despite important progress in direct Becker/Duchenne muscular Dystrophy (B/DMD) diagnosis, genetic counselling is occasionally limited when the causative mutation could not be identified. In such circumstances, indirect diagnosis is the only one feasible but can also be seriously hampered by the huge size of the B/DMD locus and its high recombination rates. This is particularly true in the 3' second part of the DMD gene where only few markers of low informativity are available. All of these drawbacks are illustrated in the two families we report here: index case deceased without available material, high Bayesian estimated risk for the female relatives and absence of informativeness of the markers located in the 3' region of the B/DMD locus. To solve the genetic counselling in both families we performed a systematic search of short tandem repeat sequences in the 3' region to detect novel polymorphic markers. Three sequences were selected: in intron 67 (IVS-67), intron 76 (IVS-76) and in the 3' untranslated region, 250kb downstream of the stop codon (3'-UTR). Labelled primers DNA were designed to amplify these sequences from DNA of 78 control subjects given a total of 108 chromosomes that were studied with standard procedures. Observed allele numbering and frequencies allowed inferring each marker's informativeness with calculation of the heterozygosity and the Polymorphic Information Content (> 0.6). Their use allows to increase the reliability of the haplotypic analysis in the two families and to reasonably exclude risk in the female relatives. These markers provide new tools for efficient indirect diagnosis of B/DMD.

P1359. The Iranian Human Mutation Database

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Regional genetic databases provide scientists all over the world with the valuable resource of specific information about the genetic structure of people from particular regions of the world in order to promote the diagnosis, treatment and basic research of genetic disorders. The Iranian population consists of different races, tribes and religions and has a highly heterogeneous gene pool and mutation spectrum, which, in many cases, can also be extrapolated to the mutation spectrum of people in the neighboring countries within the Middle East. The Iranian Human Mutation Database (IHMD), established in February 2004, is a collection of information about reported (published or submitted) mutations and related polymorphisms found within the Iranian population. We have created it to make this wealth of genetic data more available to researchers, healthcare providers and to patients and their families. The IHMD is accessible through the World Wide Web at: <http://www.uswr.ac.ir>. So far, data have been submitted regarding more than 392 mutations related to 95 genetic diseases with distinct genes. References and authors are also listed by the mutation.

P1360. Results of the Medical Genetics services in the South-Western part of Romania

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The results of medical genetics investigations in the population of the South-Western part of Romania in the last 5 years are presented. Individuals were referred to the Medical Genetics services from Timisoara by medical personnel or came without any referral. Familial pedigree analysis, clinical, paraclinical and cytogenetic investigation revealed:

- A genetic cause in the majority of investigated couples with sterility and infertility.
- Androgen insensitivity and congenital adrenal hyperplasia as the prevalent causes of intersexualities.
- Turner syndrome and its variants followed by Klinefelter syndrome as the cytogenetic causes of hypogonadism.
- Considering single-gene disorders, different types of osteochondrodysplasia were predominant, followed by storage disorders and cystic fibrosis. Due the severity of the phenotype and the psychological impact of the defects, rare disorders were diagnosed: Floating-Harbor and Ambras syndromes.
- As we expected, the main cause of mental retardation was Down syndrome, followed by single-gene disorders.
- Among non-syndromic birth defects, limb defects were most frequent.
- The majority of hematological malignancies were CML, followed by acute leukemias. Conventional cytogenetic analysis was used for monitoring these patients every 6 months. Conclusions: Couples with sterility and infertility represented the majority of investigated persons, their number increasing every year. The vast majority of patients came for cytogenetic analysis. An important percent of persons came without any referral, knowing about the genetic services from mass-media or web-sites.

P1361. The Nowgen Network: an on-line system to promote national and international collaboration within the human genetics field

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The field of human genetics clearly benefits from the exchange of information and the thorough debate of new developments. However, due to the rapid evolution of the area, there are problems associated with the identification and dissemination of information and with the practicalities of debate with interested parties. To address these issues, Nowgen and its partner MerseyBIO have worked together to develop a technologically advanced website. Within the site, virtual communities of experts have been formed to support inter-institutional and interdisciplinary genetics initiatives. Members of the Nowgen team work closely with these "Nowgen Network" communities to support their use of the system. Initially, a number of pilot communities have been established to focus on clinical, ethical, legal, scientific and educational issues associated with genetics. From a single point of access, users can utilise powerful web-based tools, allowing them to identify partners, obtain expert advice, debate issues and retrieve personalised, current information. Automatic information retrieval tools use advanced software that can search web pages and many different file types. Community interactions are supported by features that allow on-line collaboration, including multi-user, real-time conferencing and chat facilities. Importantly, the website can accelerate information retrieval, support collaborations and debate without geographical limitations and facilitate the dissemination of knowledge in support of the worldwide genetics community.

P1362. Genetic education. Acceptance to testing for genetic predisposition - part 3 - The Final

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This presentation is the third closing part of five-year study over attitude of Bulgarian people towards the possibilities and achievements of genetic science.

The first part, that included women, health-care workers (non-genetics) was "Acceptance of testing for genetic predisposition to breast cancer: 1.The attitude of medical professionals."

The second part covered the opinion of medical or biological students and was titled "Acceptance of testing for genetic predisposition: 2.The attitude of academical youth to PS DNA t for autosomal dominant inheritable late onset diseases.

At this third level we expanded our study in the base genetic acknowledgments of pupils from specialized in biology high schools and their tutors.

In conclusion this study compares the three groups - doctors, students and pupils mainly towards:

1. Information sources
2. Level of attractiveness of the genetics as a field for future work
3. Personal choice in genetic case situation

P1363. Lack of evidence for association of the endothelial nitric oxide synthase gene polymorphisms 4VNTR and Glu298Asp and idiopathic recurrent miscarriage.

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Lack of endothelium-derived nitric oxide is associated with vasospasm and vascular infarction. In the present study we investigated the relationship between idiopathic recurrent miscarriage and two polymorphisms (4VNTR and Glu298Asp) of the gene encoding for endothelial nitric oxide synthase (ENOS). In a prospective case-control study, 126 women with idiopathic recurrent miscarriage and 161 healthy controls were studied. We used the PCR method to identify the different alleles of the intron 4 VNTR polymorphism of ENOS gene and PCR-RFLPs method to genotype the individuals for the Glu298Asp polymorphism of the same gene. For the 4VNTR polymorphism the frequencies of bb, ab, aa, were 0.75, 0.24, 0.01 in the patient group and 0.73, 0.24, 0.03 in the control group, respectively. For the Glu298Asp polymorphism the frequencies of the three genotypes (GG, GT, TT) were 0.42, 0.45, 0.13, respectively, for the patient group and 0.49, 0.46, 0.04, for the control group. The data between the two groups were analyzed by chi-square test.

We found no significant differences in the frequencies between the patient group and the control group for both the studied polymorphisms. Conversely to previous results by others, the data of this work do not support a role for the 4VNTR or Glu298Asp polymorphisms of ENOS gene as a genetic determinants of the risk of idiopathic recurrent miscarriage.

P1364. Molecular study in Brazilian cochlear implant recipients

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The most common form of non-syndromic autosomal recessive deafness is caused by mutations in the GJB2 gene (encoding connexin 26). Recently, a deletion truncating the GJB6 gene (encoding connexin 30), near GJB2, called del (GJB6-D13S1830) has also been described normally accompanying mutations in another allele of the GJB2 gene. Amongst all the mutations described to date, 35delG in the GJB2 gene is the most common and has been found in virtually all of the populations studied. Preliminary data suggest that pathologic changes due to GJB2 mutations do not affect the spiral ganglion cells, which are the site of stimulation of the cochlear implant. Besides, the survival of the spiral ganglion cells is believed to be an important determinant of the outcome after surgery. Therefore, we have studied 42 nonsyndromic deaf patients with unknown etiologies in order to determine the prevalence of GJB2 and GJB6 gene mutations in patients undergoing cochlear implantation surgery. As a result, we found 13 individuals with GJB2 mutation including two new mutations in the gene (W172X and K168R), and one patient homozygous for the del(GJB6-D13S1830) mutation. In a follow-up study, the cochlear implant patients with positive mutations will be compared to a group of deaf individuals with unknown etiologies aiming at analysing the

performance of speech regarding near future studies. Concluding, these results establish that genetic screening can provide an etiologic diagnosis, which highlights a counseling importance, and may provide a prognostic on performance after cochlear implantation, as has been hypothesized in previous studies.

P1365. Unstable aberrations as biomarker of exposure in workers handling X-rays machines

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Biologic effects seen after moderate and low doses of ionizing radiation are almost invariably the result of damage to the genetic apparatus. Workers, including those handling X-rays machines as part of their jobs are routinely monitored. The chromosomal aberrations analysis of peripheral blood lymphocytes could be an important source of information for cytogenetic injury following occupationally exposure to ionizing radiations. Chromosome breaks can interact pairwise in an exchange in an asymmetric fashion to form dicentrics, rings, and interstitial deletions.

Blood samples were collected from 40 workers and chromosomal aberrations were analyzed from 500 metaphases per person. The results were compared with those obtained from 60 control individuals selected to match at least the age and sex and who had never been occupationally or therapeutically exposed to ionizing radiation, non-ionizing radiation or chemical mutagens. The total frequency of chromosomal aberrations and the frequency of dicentrics and acentric fragments were higher in the exposed group than in controls. Dicentrics were not recorded in 29 occupationally exposed people within the imposed number of scored metaphases. The highest value of 2 dicentrics in 500 metaphases was found in one case. Dicentrics frequency was not directly correlated with the duration of employment in the exposed group.

Analysis of chromosomal aberrations could be used in the assessment of possible damages to cell genome that may occur in occupational exposure to ionizing radiation.

Group	No. cells	Chromatid aberrations (% ± SD)	Chromosomal aberrations (% ± SD) Total breaks dic ace
Control	30,000	1.54 ± 0.07	0.53 ± 0.03 0.11 ± 0.02 0.01 ± 0.006 0.23 ± 0.03
Exposed	20,000	1.59 ± 0.09	1.38 ± 0.08 0.33 ± 0.04 0.07 ± 0.02 0.98 ± 0.07

P1366. 'Three years of genetic counseling experience in Firat University Medical Genetics Department'

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Genetic counseling is the communication of information and advice about inherited conditions and a person seeking such advice is called a consultand. Our basic principle for genetic counseling process is to collect the patients or consultands data, illuminate the consultands without being directive, thus, provide making their own decisions. In the light of this principle, the main objective of this study is to determine the factors affecting their decisions and the harmony of the consultands with process and counselor, to achieve the data to reveal counseling feasibility and finally, share this experiences with other counselors.

One hundred sixty seven consultands who were referred to our medical genetics department for genetic counseling from July 2001 to May 2004, were taken into the study.

We have analyzed the data for parameters such as gender, referral status, number of sessions, satisfaction, harmony and consent for prenatal diagnosis and selective termination.

It has been evaluated the status of harmony of the consultands using a questionnaire including accepted criteria, 89.8 % of the consultands seemed to be harmonious with the counselor and his explanations, but 10.2 % of the consultands were inharmonious.

In this study, we have also evaluated the consultands' information status and affecting factors about their condition before their genetic counseling sessions. We have determined that 61.7% of the consultands had no information, 24.6 % of them partially and 13.8% of them fully informed. It has been emphasized striking findings about our genetic counseling experience

P1367. The Italian External Quality Assessment in molecular genetic testing coordinated by the Istituto Superiore di Sanità

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During recent years, there has been a growing interest in quality assurance of genetic testing. There are several EQA schemes funded either by international groups or by national governments or by private subscription(1). The Italian External Quality Assessment (IEQA) in molecular genetic testing is financially supported by the National Health System and coordinated by the Istituto Superiore di Sanità (ISS).

Public Laboratories have been enrolled, covering all Italian regions and participation is voluntary(2). The IEQA scheme covers Cystic Fibrosis, Beta-Thalassemia and Fragile-X, for which gene testing is requested very often in Italian laboratories,(3) and the adenomatous polyposis coli gene.

Four trials have been performed and the number of participating laboratories has been increasing (41, 50, 56 in 2001, 2002, 2003 respectively; 2004 trial ongoing).

Laboratories have to i) test, for each disease, six validated samples ii) send back to ISS, within two months, raw data, interpretation of results and a final written report.

For each disease, a panel of national experts evaluated all data.

Overall the evaluation reveals that analytical accuracy is good; however, written reports were not homogenous and often missing important information. A standard report format has been proposed by consensus by the ISS and national experts. An overview of all the results will be illustrated.

(1) Ibarreta D. et al., *Nat.Biot.* 2004

(2) Taruscio D. et al., *Clin Chem Lab Med* 2004.

(3) Dallapiccola et al., *Analysis* 2/3. 2004

This work is funded by the Project "Test genetici: dalla ricerca alla clinica", Italian Ministry of Health (2003-2005).

P1368. An External Quality Assessment scheme for genetic testing of Fragile X syndrome.

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Fragile X syndrome (FRAXA) is the most common cause of inherited mental retardation with an incidence estimated at 1 in 4000-9000 males and 1 in 7000-15000 females. The most frequent mutation seen in FRAXA is an expansion of a CGG repeat in FMR1 gene. Genetic testing for this expansion is routinely offered in diagnostic molecular genetic laboratories. In 2001, a pilot External Quality Assessment (EQA) scheme for FRAXA was offered by the European Molecular Genetic Network (EMQN) and 16 laboratories from 16 countries applied to take part. Since this successful trial, the number of participants has increased each year with 54 participants from 22 countries in 2004. A wide variety of methods is used and two different marking criteria were applied for the laboratories which perform the full genotyping (usually by Southern blot) and the laboratories which only perform a pre-screening by PCR method. Each year 3 DNA samples were sent out for analysis, some of the cases were chosen in order to give the opportunity to the participants to appreciate particular problems in genotyping. Error rates were calculated from the total number of alleles analysed by all laboratories. The scheme will be described in full and the results of both genotyping and interpretation will be discussed. Lessons drawn from the development of the scheme should be useful for laboratories involved in this field.

P1369. Intrafamilial variability in autosomal dominant tibial aplasia with ectrodactyly.

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Aplasia of the tibia with ectrodactyly (OMIM 119100) is a rare malformation. In most families this disorder follows an autosomal dominant pattern of inheritance. The phenotype of affected individuals

within a family is highly variable and even obligate gene-carriers without symptoms are described. This markedly reduced penetrance makes counselling difficult since the cause of the disorder is still unknown and no diagnostic test is available. Here we report on a large Iraqi family with affected members in at least 3 generations. We describe and illustrate the variability of phenotypical and radiological findings. The most severe affected person has bilateral tibial aplasia with clubfoot deformity and oligodactyly but normal upper limbs. Milder affected individuals have uni- or bilateral split hands with or without hypoplastic big toes. The mildest manifestation reported in this family was an individual with abnormal big toes only. In addition an obligate carrier without clinical symptoms who has 3 affected children was identified. As a consequence, regarding genetic counselling we suggest to evaluate the family history very carefully in patients with only minimal symptoms like short big toes. Furthermore, if an apparently unaffected person has an affected parent or if one of his parents can be assumed to be a gene-carrier, the risk for an affected child is maximally 8.6% per se [Majewski et al., *Hum Genet* 70:136-147, 1985] but could be 50% at worst. Therefore, prenatal ultrasound examination is important also in unaffected parents with that familial constellation.

P1370. Cystic Fibrosis: an interactive educational software for teaching the disease

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Cystic Fibrosis (CF) is a severe chronic autosomal recessive disease, with an incidence of up to 1:2000 in Caucasian populations. Since Brazil is a multiracial mixed population, we have investigated the notification of CF as primary cause of death in Brazil. We came to the conclusion that CF was underestimated mainly in inland parts of the country. One explanation was that CF symptoms, such as pulmonary infection, diarrhea and malnutrition, were also common causes of death of non-CF infants in Brazil; and another possibility was that the physicians working in the interior of Brazil were not acquainted with the disease. In order to divulge the disease, we have developed easy educational software, in Portuguese language, for teaching the signs, symptoms and the molecular basis of the disease. For that, we have developed an interactive software using the following programs: Microsoft Power Point, Corel Trace, Corel Draw, WindowsMetaFile and Flash Player version 5.0 r 30 (Macromedia, Inc.). The software was totally produced by a group of six undergraduate medical students as an activity of the Molecular Biology Course. The presentation, in Power Point, had 19 slides with theoretical text on CF, x-ray images of normal and CF lungs, microscopic images of CF pancreas and three original interactive animations showing the mechanism of action of CFTR (Cystic Fibrosis transmembrane conductance regulator) protein. Therefore, the slide show saved on CD disc can be easily replicated and used for teaching transport across cell membrane and also as divulgation of CF to Health Care professionals.

P1371. The European Skeletal Dysplasia Network (ESDN)

- A model for increasing access to genetic testing of rare conditions.

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Skeletal dysplasias are a diverse group of genetic diseases affecting the development of the osseous skeleton. Because skeletal dysplasias are rare and exhibit extensive clinical variability and genetic heterogeneity, accurate diagnosis is a challenge for the non-expert. To provide equity of access to diagnostic experts we have established the European Skeletal Dysplasia Network (ESDN). The ESDN has

adopted two approaches; the research component of the project focuses on identifying the genes, mutations and disease processes that underlie skeletal dysplasias, whilst the diagnostic component integrates a network of expert clinicians and laboratories. Since January 2002 the ESDN has received 1513 patient referrals from 23 EU and 10 non-EU countries. The causative mutations have been identified in 503 patients following 1066 diagnostic tests. Furthermore, the development of a custom built secure web-based case management system allows clinicians to refer cases to the ESDN from anywhere in the world. Through the ESDN Case Manager, a clinical description and x-rays are assessed by the ESDN's panel of expert reviewers and an initial clinical diagnosis is confirmed or suggested. Patient DNA samples are then sent to the appropriate ESDN partner laboratory for molecular diagnosis. This is the first pan-European approach to the diagnosis of rare diseases, and therefore has major implications for the delivery of diagnostic services in the EC for the 21st Century. The model established by the ESDN is applicable to the diagnosis and management of any group of rare disease.

www.ESDN.org

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P1372. The Canadian Molecular Cytogenetics Platform : Developing Informatics Security and Access Resources

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The Canadian Molecular Cytogenetics Platform (CMCP) is an infrastructure partnership of 13 major research facilities that supports the research of leading clinical investigators and basic scientists. For example one of its projects, Genomic Tools for Diagnosis and Evaluation of Mental Retardation, aims to evaluate high-resolution BAC microarray comparative genomic hybridisation as an alternative method of identification of chromosomal abnormalities in individuals with mental retardation. It will compare samples from affected individuals, mostly children, and their family members with those from an ethnically diverse group of unaffected adults.

The CMCP is building a system for the collection, storage and sharing of clinical and research data and specimens from 18 centres throughout Canada. Before research is undertaken, an Informatics Security and Access Platform is being developed by the Genetics and Society Project of the Centre de recherche en droit public at the Université de Montréal. Its goal is to ensure that the research supported by the CMCP is conducted in accordance with current legal and ethical standards. Can such a collaborative network creating both local and centralised databases adequately protect participants and yet ensure the highest scientific quality? Beginning with an analysis of potential risks with regard to confidentiality, integrity, and availability of data, the informatics access and security resource is defining the necessary safeguards for samples and data. A code of conduct, of principles and policies; standard operating procedures; conflict of interest and copyright/intellectual property guidelines; and an informed consent template and process are being developed.

P1373. St.Petersburg society of the parents with PKU children (ORDI-PKU)

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In memoriam Irina V. Butomo

The society of the parents with PKU children (ORDI-PKU) was founded in 2002 in Saint-Petersburg, Russia. Cooperation of PKU families is a very important fact in St.Peterburg history of PKU. The main aim of ORDI-PKU is to create adaptation system for children with PKU, to coordinate parents activity and to make a bridge between PKU families and geneticists and other physicians. Only fifty two families with PKU children are members of our society. Only 30% of them are the active members and participants of the different projects of our society. Our close cooperation with the Center of Medical Genetics, the Department of Medical Genetics of the Medical Academy for Postgraduate Training ensures modern medical and diet management. We have financial support for purchase different Phe-free powders. All children with PKU are under geneticists observation, this medical care is free of charge. Unfortunately, there is no possibility to hospitalize an infant

with PKU for initiation dietary management. There is no solution of the serious problem of the atypical and maternal PKU in St.Petersburg. Maintaining lifelong compliance with Phe-free formula is not discussed. Amino acid analysis of high quality and DNA analysis for confirming PKU diagnosis are rare and very expensive. There is no constant team recruited different specialists, including dietician, for long-term contact. Some parents and probands do not understand PKU problems clearly and accurately. To have own web site, newsletter, modern handbooks, booklets, hot-phone number is the requisite condition of successful management of PKU families

P1374. Inheritance Pattern in Congenital Heart Disease

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Background: About 0.8% of live births are complicated by cardiovascular malformations. Most of these anomalies appear to have a multifactorial or polygenic etiology. About 5-7% of cardiovascular malformations result from a chromosomal defect and about 3% of them has a single gene disorder. The aim of this study is to determine genetic patterns influencing congenital heart disease (CHD).

Materials & Methods: We studied 203 consecutive patients suffered from CHD being referred to cardiovascular research center during 10 months. Familial pedigree, Clinical and paraclinical data of patients were evaluated.

Results: There were 90 male and 113 female in this study (mean age = 5.48). The most common malformations were VSD (19.2%), ASD (11.8%), PDA (11.2%) and TOF (10.3%). Of these patients, 70 patients had a positive familiar pedigree with CHD. About 71% were born from consanguineous marriage of different degrees (76% third degree). In 67% of cases at least one more relatives had the same problem while in one family another 4 relatives found to have CHD. The highest inbreeding coefficient (13.28) was found in one family and 39.9% had 6.25=<F. The inheritance pattern in this patients included Autosomal Dominant (5.7%), Autosomal Recessive (5.7%), Sex Linked (2.9%) and positive pedigree without inheritance pattern (86%).

Conclusion: The result can help to physicians and genetic counselor to realize the contribution of inheritance pattern in congenital heart disease and in to recognition and prevention of subsequent CHD and in setting priorities of screening in individual cases.

P1375. The Inheritance Pattern of Blind People Supported by Rehabilitation Organization

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Objective: Visual loss is one of the most important of infirmities, affecting about 514000 people in Iran. The aim of this study was to explore the frequency of the types of blindness and the role of inheritance patterns implicating autosomal and X-linked disorders in this.

Materials & Methods: In this clinical and cross sectional study, a genetic counseling program was performed for patients that were supported by the Yazd Social Welfare and Rehabilitation Organization. The subjects were referred to Genetic Counseling Center during 9 months by Yazd Blind's N.G.O. Based on genetic counseling, familial pedigree and other clinical and paraclinical data, containing ophthalmologic assessments were accumulated.

Results: In 109 patients, 73 and 36 patients were males and females, respectively. The mean age was 24.62 (SD=10.49). In these patients, 66 had a positive pedigree of blindness. 66.1% (72 patients) were born from a consanguineous marriage in different degrees (58.1% in third degree). There was inheritance pattern in these cases with autosomal dominant (10.6%), autosomal recessive (56.1%), sex linked (15.1%), positive pedigree without inheritance pattern (18.2%). There were 13 blinds in familial pedigree as autosomal dominant inheritance in one proband. The most common types of blindness were retinitis pigmentosa (32.1%), and microphthalmia (16.5%).

Conclusion: Familial marriage was related to in expression of blindness in our patients. (P = 0.00 & odds ratio = 2.75). The results provide information for physicians and genetic counselor to realize contribution of inheritance pattern in blindness.

P1376. Thalassemia and Prenatal Diagnosis: Example of Iran, prevention program & its peculiarities

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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, religious and official frameworks to deal with the aftermath of such a diagnosis; i.e. abortion therapy were not sanctioned yet. β -thalassemia with over 15000 registered cases, 5000 more non- registered cases, estimate of three million carriers, expensive treatments and stigmas and psychosocial problems made it an obvious target for prevention. A thalassemia prevention program was in action since early 1990s. The aim of that program was to identify the at-risk carrier couples by blood testing premaritally and persuade them not to get married. Nevertheless, the outcome was not satisfactory : less than a third of the couples accepted the recommendation and the majority went ahead with their marriage options. So, the risk of affected newborns remained, and ineffectiveness of the program made itself apparent. This was because it lacked the offer of PND to the at-risk couples. This defect was finally rectified in 1997 and the offer of PND to the at-risk couples was incorporated in the program. Adoption of such a policy has paved the way for a more comprehensive approach in prevention of genetic disorders in general. This article provides an account of the past and present situation of prevention program for genetic disorders in Iran.

P1377. Estonian Womens' Attitude to Prenatal Diagnosis

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Aim: To investigate pregnant womens' experience prenatal counselling in connection with prenatal diagnosis (PND).

Methods: We gave questionnaires to two group of pregnant women: group A - women aged 37 years and over; group B - women under 37 years who had received II trimester biochemical serum screen-positive result.

Results: Most of the women of group A and half of group B knew about PND before pregnancy. The main source of information had been gynaecologists and midwives. The main reasons for having an invasive procedure were the assurance for having healthy baby and the doctor's advice. The women in group A were more satisfied with the information given at genetic counselling, than group B. 99% of women in group A and 71% in group B knew about methods and risks of PND. All women in group A and 91% in group B asked for genetic counselling. The main feelings after the procedure was satisfaction with decision and that the procedure wasn't so harmful. Most of the women said that they would undergo PND during another pregnancy. At the time of the test, more than half of participants (55%) would choose a legal abortion if the test indicated an abnormality in the foetus.

Conclusion: Women in the age risk group are satisfactorily informed probably because PND is well known among them. For group B PND came unexpectedly and it is most probably difficult to cooperate for amniocentesis because of fear. However all women were sure, that prenatal diagnosis was necessary.

P1378. Quality Management and Quality Assurance in Molecular Genetic Testing Laboratories.

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Genetic testing services in Europe have substantially increased their activity in the past few years. Testing for genetic diseases has rapidly moved from the laboratory into medical practice and, in this process, issues of quality require adequate attention. The organization of annual external quality assessment schemes in Europe, complemented by regional workshops on quality, has demonstrated that quality assurance is essential in order to minimize errors in genetic services. It has been conclusively shown that errors and deficiencies can occur everywhere

during the processes of sampling, genetic testing, interpretation, and reporting of results.

The introduction of international standards for quality and competence in laboratories, together with a growing interest in accreditation of laboratories, has increased the need for a better understanding of quality management and quality assurance. The International Organization for Standards (ISO) defines quality as 'the degree to which a set of inherent characteristics fulfils requirements'. The EUROGENTEST network aims to help and guide medical genetic laboratories in order to better understand this type of 'standards language' that is used by accreditation bodies.

An educative website will be constructed with links for each EU country to relevant information on accreditation bodies, contact persons, coordinates of the national standardization institutes, and explanations of terminologies. Furthermore, a database will be prepared to publicize with relevant information on quality assurance in European laboratories. Finally, a study is planned to calculate the 'real cost' for implementing and maintaining a quality management system in a genetic service laboratory.

P1379. Telegenética: an online consultation service for health professionals.

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Telegenetica is an online consultation service in clinical genetics open to registered professionals who seek diagnostic support or specific information for a patient, in spanish. The website contains specific forms to guide non-genetics specialists in clinical interview and examination. The consultations are directly replied by two certified genetics specialists. For those cases without a firm diagnosis, the patients are included in an online discussion board of collaborating genetics specialists. The program is directly linked to Orphanet, the European online database in rare disorders, coordinated in Spain by this same group. During the initial 18 months of Telegenetica, 179 health professionals have registered: 142 physicians, 14 biologists, 3 pharmacists, 3 teachers, 2 psychologists and 2 nurses. Pediatricians, obstetricians, geneticists and family doctors submitted most consultations. The service is presented to the patient as a confidential and anonymous specialist consultation, but the referring doctor is responsible for providing genetic counselling or referring the patient to a Clinical Genetics service. A specific diagnosis has been established for over 50% of cases submitted. A satisfaction questionnaire for participating professionals and patients, and a pilot study for the blind evaluation of the patients referred online has been completed and reflects high levels of diagnostic efficiency, as well as physician and patient satisfaction. The referring physicians perceived this service as a diagnostic support but also as a valuable educational tool for acquisition of basic knowledge in clinical genetics. The specialists discussion board has provided new diagnoses and fulfilled the educational expectations of the participants.

P1380. Is consent required for immunohistochemistry and microsatellite instability testing of tumour samples?

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One central purpose of the informed consent process in the delivery of genetic testing within clinical services is to help ensure that the client understands the personal, medical and familial implications of performing the test. Written consent is a prerequisite for cancer genetic testing and is usually provided after genetic counselling.

Immunochemistry for mismatch repair proteins (IHC) and microsatellite instability (MSI) testing of tumours suggest if mismatch repair gene defects contributed to a family history of HNPCC-related cancers and are particularly useful for assessment of families who do not meet the modified Amsterdam criteria. Therefore, they may be considered screening tests as they identify a subgroup of individuals eligible for diagnostic genetic testing. The All Wales Cancer Genetic Service provides IHC and MSI testing after genetic counselling and written consent is obtained.

It is not feasible for genetic services to see all individuals who may be eligible for MSI/IHC. Testing could be initiated by a surgeon on the basis of suggestive family history, by a pathologist for tumours showing features consistent with an HNPCC phenotype or as part of a program to test all colorectal tumours diagnosed under a specified age. The consent process in each of these settings differs from standard practice in genetics.

We consider the practice and purpose of consent in each of these settings. We ask if there should be consistency in the consent process, regardless of the context in which MSI and IHC are initiated, or if the different settings require different approaches to consent.

P1381. Education, Genetic Counselling & Congenital Malformations in Iran

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The central place in bioethical decisions actually belongs to the patient. The research was undertaken studying 800 cases from different areas of Tehran in two year's span of data collection with the aim of studying the role of education of the parents in preventing the spread of certain genetic disorders in Iranian population. And also to study the present position of genetic counseling in Iran and their utility by people.

Analysis of data on educational level of the patient's parents who consulted genetic clinics revealed that around 41% had secondary level of education. 15% were well educated while 12% were illiterates. Majority (98.8%) of them referred to these clinics by their consulting doctors. 1.2% who went on their own had certain level of education, majority were well educated and professionals.

In one of the main genetic centers, a total of 2246 patients came for genetic counseling before marriage and mostly were related. Nearly 23% came for reconfirming the abnormality of their affected child. 16% came for having repeated abortions. 15% came because of being infertile and wanted to know if there is any genetic cause behind that. 12% came for counseling as they already had an affected child and wanted to have another child again.

Central to prevention is the recognition of causes, which in Iran emerges out to be predominantly consanguinity in Marriage partners. Best of programs are bound to fail if they lack attention to development of positive attitudes towards prevention in both the people and the professionals.

P1382. BRCA analysis - pitfall in finding the index patient

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We report on a family with breast cancer affected members in two generations. The usual procedure after respective genetic counselling is to test an "index patient" within the family who is an affected first or second degree relative of the risk asking woman. Our chosen index patient (maternal aunt to risk asking woman) who had breast cancer at age 49 years showed no mutation in the BRCA1/2 genes. In concordance with the usual German genetic test criteria we offered no further analyses in this family. A few months later the younger sister of our risk asking woman was diagnosed with breast cancer at the age of 45 years. She is the first affected family member in the third generation. We decided to offer a gene test although no mutation was found in the first index patient. Surprisingly we found a mutation in the BRCA2 gene. A subsequent analysis in our risk asking woman showed the same mutation. Since the father of the sisters who is still alive does not carry the mutation it must have been inherited maternally. Therefore, the breast cancer disease in our first analysed index patient seems not to be related to the inherited form of breast cancer in this family. Because of this pitfall in choosing the "right" index patient it seems to be of interest to discuss whether the German genetic test criteria should be extended in the sense of testing every affected family member.

P1383. Diagnostic procedure for the assessment of hereditary prosopagnosia

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Prosopagnosia is a selective impairment in the visual learning and recognition of faces. There is a hereditary type of prosopagnosia without any detectable brain damage or malformation, which has an autosomal dominant mode of inheritance. It is the only known hereditary disorder of higher visual cognitive functions. There has been no standard diagnostic procedure so far for this disorder, because most researchers only saw single cases. We assessed 36 cases of hereditary prosopagnosia in order to develop a standard diagnostic procedure. We compiled a checklist of symptoms in three groups: Group one contains six leading symptoms, where at least five must be present. Group two contains five facultative symptoms, of which at least two must be present. Group three is a list of symptoms, which are usually not associated with isolated prosopagnosia. If three or more of these symptoms in the latter group are present, a face recognition deficit is probably not caused by hereditary prosopagnosia. The diagnostic procedure has been verified independently by other researchers. It could be shown, that the subjective symptoms are indeed accompanied by an objective face recognition deficit. This is the first diagnostic checklist for the hereditary prosopagnosia and can help to establish a standard diagnostic procedure for this common hereditary disorder.

P1384. Biological Effects and Health Risks of Cell Phone Radiation

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Concerns about non ionizing radiation have been reported, especially considering possible biological effects and health risks to individuals exposed to this sort of radiation. The objective of this study was to accomplish a critical and systematical review of published articles and to analyze, qualitatively, the *in vitro*, *in vivo* and epidemiological results about the effects of the non ionizing radiation in cells, individuals and populations. Two hundred and twenty one Medline-indexed scientific articles were reviewed. *In vitro* findings reported DNA and protein expression damage, increasing cellular proliferation as well as the increase in aneuploidy of certain chromosomes and cell division asynchrony even that the majority of results are yet conflicting. DNA damage was also verified by the REFLEX study (in a joint effort from European countries) and is also supported by our study considering effects in cells and tissues caused by non ionizing radiation. Epidemiological studies pointed to other effects, as headaches, sleeplessness, dizziness and cognitive effects, which were found, also, in *in vivo* studies. Some studies suggest that this kind of radiation might induce acoustic neuromas (a benign tumor). Although there are plenty of data about the problem, few conclusions could be delineated. It was proved that radiation induces a biological response but it is still unclear if it is a real health risk. As the radiation problem is still new for the population to know its effects, users and governments should take a precautionary approach until its major effects could be finally understood.

SUPPORT: FUNTTEL

P1385. 'To know yet not to know': preimplantation genetic diagnosis of inherited prion diseases

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Genetic Creutzfeldt-Jakob disease (CJD) is a fatal degenerative brain disorder with autosomal dominant inheritance and near 100% penetrance. Jews of Libyan origin have an increased incidence of CJD due to a common founder mutation. We report preimplantation genetic diagnosis (PGD) by exclusion for CJD in embryos from a daughter of an affected patient. The daughter did not wish to learn her carrier status, yet wanted to have non-carrier children. Following an IVF procedure, embryos that inherited the non-affected parent's haplotype were identified and transferred. PGD by exclusion for CJD provides a

non routine option for individuals who wish to avoid the transmission of the mutant gene without revealing their own carrier status.

PGD by exclusion is a significant option in light of potential discrimination and fear of stigmatization of the Libyan Jewish community where the incidence of CJD is 100 times higher than worldwide incidence (50 per million as opposed to 0.5 per million). By implementing such technology these families may be able to halt the transmission of this devastating, non-treatable disease and to avoid ethical dilemmas concerning predictive testing.

P1386. Developing curricula based on learning needs: genetics education for specialist registrars in non-genetics specialties.

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Background: In June 2003 the Department of Health, England, published a White Paper, "Our Inheritance, Our Future", setting out a strategy to prepare the NHS for changes in healthcare that could result from the Human Genome Project. It recognises a need for improved genetics training at all levels of medical education.

Summary of Work: The study aim was to develop a genetics curriculum for specialist registrars in dermatology, cardiology and neurology. Curriculum development was informed by three sets of data. Firstly, a mapping exercise of current genetics education, including curricula analysis, interviews with educators, and a survey of specialist registrars in the selected specialties. Secondly, focus groups with specialist registrars in the selected specialties in the West Midlands and South Western deaneries. Thirdly, an online modified Delphi survey of a national sample of consultant geneticists and specialty consultants.

Summary of Results: Collection of the three sets of data enabled an evaluation of the synergy between current teaching of genetics up to SpR grade and their identified learning needs in the modern health service. Priority areas for teaching and learning have been identified and strategies for effective delivery developed.

Conclusions: Curriculum development is often based on the opinions of a small number of experts. This project demonstrates an alternative model, in which curriculum development draws on a wide range of data sources. Such an approach has highlighted the need for genetics education for specialist registrars to be based on learning needs, focused on agreed priorities, and made relevant to each specialty.

P1387. Genetic services in public health

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Purpose: the improvement of public health in field of medical genetics.

Methods. Genetic monitoring system works since 1999, it consists of territorial (Kyiv and Kyivska region) registers of newborns birth defects (2840 cases), spontaneous abortions (3363 cases), sterile marriages (1268 cases, sterilitas I). Risk factors influence was estimated by odds ratio (OR) with 95% confidential interval.

Results. The number of patients for genetic consulting has increased 1,5 times during the studied period. Time for congenital pathology diagnostics decreased. It was find out that parents infectious diseases increase probability of birth defects among newborns, spontaneous abortions, sterile marriages (OR=3,42 (2,38-4,97); OR=4,56 (3,23-6,37); OR=17,79 (12,31-24,50) correspondingly). Endocrine pathology also increases such risks: OR=3,17 (2,26-4,45); 2,78 (1,93-4,00); 5,78 (3,84-8,68) correspondingly.

It was detected that spontaneous abortions (till 12 weeks of gestation) risk increases of women live in the radioactive polluted regions of Kyivska region (OR=1,36 (1,09-1,71), and mechanisms of this phenomenon should be investigated.

Conclusions. Concerted action of doctors, health care managers and scientists within genetic monitoring system allowed to decrease foetus reproductive losses risk 1,36 times (1999-2003 in comparison with 1994-1998): spontaneous abortions rate decreased 1,45 times, stillbirth rate and early neonatal (first 6 days) death rate decreased 1,12 and 1,48 times correspondingly.

P1388. Genetic counselling for thalassemia and haemoglobinopathies for immigrants in Emilia-Romagna and north-east Italy

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Immigration in the European Community, either from countries of other continents or from other part of Europe, implies that the national health systems should face specific problems considering the cultural differences in the ethnicities. Hemoglobinopathies, one of the most common autosomal recessive disorders in the world, is a disease to deal with preventive actions, identifying the carriers and providing genetic counselling. In the last five years, 210 counselling sessions for risk of thalassaemia and haemoglobinopathies have been performed for immigrants at the Medical Genetic Service of Ferrara, for residents in Emilia Romagna and in North Eastern Italy. This group is about 10 % of the total counselling provided for these diseases in the considered period. 59 % of immigrants are of African origin (in particular countries of Guinea Gulf and North Africa), 21% are European (mostly from Albania), 13 % are Asiatic, 7 % are from central and south America. 12% of total consultations were performed for singles, 14% for couples prior to pregnancy, 74% for couples during a pregnancy (49% of the couples of this group resulted at reproductive risk). The request for prenatal diagnosis was different between the European group and the African one: a uniform choice for prenatal diagnosis in the European group, a more articulated request in the African one. The collected data about the wide spectrum of molecular defects, the different pregnancy's time of referral to counselling and the previous knowledge of risk seem to condition differently the reproductive choices in various ethnic groups.

P1389. Do midwives think genetics is important? Implications of their views and confidence for the provision of genetics education.

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In the UK, midwives are the primary healthcare providers for women during pregnancy, childbirth and the early postnatal period. An understanding of genetics is therefore important. However, studies have shown that midwives receive little genetics education. To inform the development of relevant genetics education, this study examined midwives' views of the importance of genetics and their confidence.

A questionnaire was sent to all midwives in four maternity units in the West Midlands (N=817). Clinical scenarios covered genetic conditions relevant to midwifery practice (cystic fibrosis, Turner syndrome, Duchenne muscular dystrophy). Genetic activities were listed for each scenario, such as "Explain to Kelly how cystic fibrosis is inherited" and "Identify whether Sarah should be referred to specialist genetics services". For each, midwives rated the importance to midwifery clinical practice and their personal confidence to carry out the activity using a bipolar rating scale.

The response rate was 51%. Questions were categorised into 'clinical', 'psychosocial & ethical' and 'biological'. Midwives rated all categories important in clinical practice. However, personal confidence was rated low or fairly low. Confidence was lower for biological and clinical questions than psychosocial questions. Most (89%) were interested or very interested in genetics courses. There was no significant difference for interest in genetics education with age, clinical grade, maternity unit, education background or previous genetics education. Those with some genetics education were more interested in longer courses than others.

These findings have been used to provide genetics courses for midwives that enable them to meet the demands of their role.

P1390. The psychological effect of BRCA1/2 genetic testing: A prospective study

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The long-term psychosocial effects of genetic counseling and testing for families at high risk for developing breast/ovarian cancer are important

issues relevant for applied clinical research. 160 high risk Jewish women, were pre and post counseling evaluated regarding distress levels, coping style usage and social support. Mean age ranged from 32.7+4.7 (for the asymptomatic BRCA1/2 mutation carriers) to 54.4+12.9 (for non-carriers diagnose with breast cancer), with significant differences ($F=18.7$; $p<.001$). Ethnic origin was attributed by country of birth of the case, her parents and both sets of grandparents. Based on results of genetic testing, participants were divided into four subgroups: breast cancer-affected BRCA1/2 mutation carriers ($n=16$), breast cancer-affected non-carriers ($n=66$), asymptomatic BRCA1/2 mutation carriers ($n=16$) and asymptomatic non-carriers ($n=62$). Pre- genetic testing analysis showed that, breast cancer-affected women expressed the highest levels of distress (0.8+0.3 for mutation carriers and 0.8+0.7 for non-carriers) and social support (5.6+0.6 for mutation carriers and 5.1+0.6 for non-carriers). Whereas, post genetic testing results showed that only asymptomatic BRCA1/2 mutation carriers were effected by genetic testing as reflected in increased levels of somatization (1.3+0.9), depression (1.3+0.8), anxiety (1.3+0.8), and on the GSI (Global Severity Index) (0.8+0.8), and use the avoidance coping style (4.9+0.5). Regression analysis revealed three predictors of distress levels: breast cancer status, mutation carrier status, and use of the avoidance coping style.

These findings indicate that in Israeli asymptomatic BRCA1/2 mutation carriers, oncogenetic counseling and testing has profound psychosocial effect. This subset of individuals should be targeted in future studies aimed at stress reduction.

P1391. X-linked mental retardation Italian network

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The X-linked mental retardation Italian network includes 27 clinical centers and 9 laboratories. It collects clinical information and biological materials of mentally retarded males negative for FRAXA and chromosomal and subtelomeric rearrangements. The following XLMR genes are presently analyzed: *RSK2*, *ARX*, *IL1RAPL*, *XNP*, *OPHN1*, *ACSL4*, *AGTR2*, *FMR2*, *SLC6A8*, *MECP2*, *NLGN3*, *NLGN4*, *PQBP1*. Whenever possible, enzymatic tests or metabolite dosage are employed. If mothers' DNA is available, X-inactivation status is analyzed. In familial cases, exclusion mapping or classic linkage analyses are performed. A dedicated web site has been created for the network (<http://www.xlmr.unisi.it>). General information on the site is publicly available. Specific information can be accessed only from network members and includes: 1-Detailed clinical data; 2- Location of biological samples; 3- Laboratory results. At present, the network has collected DNA and lymphoblastoid cell lines of 103 XLMR cases (28 familial, 13 likely familial and 63 sporadic). Linkage analysis allowed to define a possible new locus in Xq27 in a family. Exclusion mapping in small families allowed to exclude several loci addressing mutation analysis to the remaining genes. Skewed X-inactivation in the mother was found in 5 sporadic and 8 familial cases, addressing mutation analysis to specific genes. Creatine blood dosage was elevated in 4 cases suggesting a *SLC6A8* mutation. Creatine dosage in urine of these patients will confirm this hypothesis. Standard mutation analysis of the other genes has been performed in 1/3 of the cases and no mutations have been found until now. Telethon (n°GTF02006) and Fondazione Mariani grants to A.R.

P1392. The NHS National Genetics Education and Development Centre: principles and challenges

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The Department of Health in England is developing a strategy to prepare for healthcare changes that could result from the Human Genome Project. Recognising a need for improved genetics training at all levels of the NHS, it has established the National Genetics Education and Development Centre to provide a focal point. The Centre has a large task ahead, but has identified key principles, designed to recognise and engage the everyday practice of healthcare

professionals. They include understanding what patients expect to find out about genetics from the different health professionals they meet, and using patients' experiences to demonstrate the clinical utility of genetics.

The Centre has a collaborative multidisciplinary team with expertise in educational theory and practice, communications, information technology, evaluation and event management. Initial work programmes centre on three NHS staff groups (medical practitioners; nurses, midwives and health visitors; and pharmacists) and focus on assessing genetics educational needs, developing and evaluating curricula, competency frameworks and educational resources; encouraging the integration of genetics into pre- and post-registration courses and continuing professional development

Recognising the need to work through the existing professional bodies, providers of training and education, and not overloading curricula, the Centre is working in close co-operation with many organisations. It is especially important to keep responsibility for provision of education within current mechanisms and providers. Through being based in a regional genetics service, we can incorporate rapid advances in genetic practice into the educational initiatives.

www.geneticseducation.nhs.uk

P1393. EuroBioBank, the leading European Network of DNA, Cell and Tissue Banks for Rare Diseases: results of the first two years of activity.

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EuroBioBank (EBB), the leading European network of DNA, Cell and Tissue Banks for Rare Diseases was established in 2000 thanks to the collaboration of 16 partners from 8 countries, in order to improve accessibility of human biological resources. The project was funded by the EC in 2002 for 3 years, under the 5th Framework Programme (QLRI-CT-2002-02769).

The expected results are: to optimise the use of existing collections and encourage the creation of new ones; to enhance collaborations in medical and scientific communities in the field of rare disorders affecting about 30 million citizens in Europe; to develop specific research and diagnostic tools; to define new therapeutic methods.

The first 2 years were dedicated to identifying and localising the existing biological material, to standardising and promoting quality banking practices, developing a network Charter, Material Transfer Agreement and Standard Operator Procedures, to distributing quality material and associated data to users.

A web site dedicated to the network activities (www.eurobiobank.org) was created with a restricted area reserved to communication and collaborations among partners of the network (EBB Intranet). Information on the EBB network has circulated through leaflets, articles in patient magazines and posters or oral communications at international congresses. Several scientific papers have also acknowledged EBB.

The EBB network is a successful, structural model for supporting scientific exchanges and cooperation. It was awarded the "Newropeans 2004 Grands Prix" for "Research and Technology", for having significantly contributed to the democratization of the European Union, closing the gap between European citizens and the EU construction.

P1394. The EMQN pilot external quality assessment (EQA) scheme for the spinocerebellar ataxias (SCAs)

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In 2004, a pilot EQA scheme for the SCAs was organized for EMQN.

This was the first EQA to deal with a group of related disorders (as opposed to single diseases or methodologies). Thirty-three labs, from 17 countries (from Europe, Israel and Canada) participated. Three mock clinical cases were used: one confirmation and one exclusion of a clinical diagnosis of MJD(/SCA3), and a presymptomatic test for SCA2 (the two most frequent spinocerebellar ataxias); 32 labs sent in their reports, and many included raw data and a brief description of methodology, as requested. There was a wide variation in size of the (normal and expanded) repeats, but only one gross genotyping error (in the PST for SCA2). Two labs determined repeat size but did not report it, while four may not have assessed it at all. We checked reports for the presence of essential items, such as major identifiers, referral information, methods, results, interpretation and recommendations, and general layout. Just 14/32 labs included all major report items, and only four respected all desirable items: one lab included only one identifier; three labs did not identify clearly both alleles; two did not have a clear analytical result; all included some kind of clinical interpretation, but seven did mention the implications for relatives, three did not recommend counselling and two did not recommend further testing, where appropriate; seven labs apposed only one signature. Some labs inappropriately genotyped other (unrequested) loci. Genotyping and interpretation scores were devised to help assess this year's full scheme.

P1395. Population genetics: governing international endeavours

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In an era where biobanking activities are developing at an increasing pace, research being undertaken not only on small cohorts for specific diseases but also at the level of whole population or communities to explain human genetic variation, the need for researchers to go beyond their national borders and collaborate in international endeavours is accrued. Several international research projects, are engaging in an effort to promote the exchange of scientific knowledge and technologies, and the advancement of genomic research. The Public Population Project in Genomics (P3G) wishes to connect leading population projects and create a common ground for exchanging and comparing datasets through the establishment of a knowledgebase for voluntary sharing of research tools with a view to possible harmonization in order to increase statistical power, cross talk between studies and validation of results.

Large-scale population biobanks pose ethical, legal and social challenges, success resting on public trust through the implementation of balanced transparent and accountable governance and monitoring structures. For international initiatives such as P3G, the international exchange of data raises multiple and complex issues. For instance, the governance structure, management and monitoring mechanisms must not only comply with national standards and policies and the structure of the individual projects involved but must also be compatible with its international vocation while ensuring public adherence. This poster will identify these and other challenges for the P3G consortium and propose potential avenues for proper transparency, accountability and monitoring of such international projects.

P1396. A sustainable community process to improve availability of appropriate, verified quality control (QC) materials for genetic testing

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The expansion of molecular genetic testing in clinical and public health practice has increased the need for appropriate, verified quality control (QC) materials for quality assurance, test validation, proficiency testing (PT), and development of new genetic tests. However, despite the growing test volume and the rapidly increasing number of tests being offered, the necessary QC materials are not available for many tests. The Centers for Disease Control and Prevention (CDC), in collaboration with members of the genetic testing community, including commercial and academic genetic testing laboratories, government agencies, professional organizations, industry, academic institutions, cell banks and proficiency testing (PT)/external Quality Assessment (EQA) programs, has developed a program to improve public availability of

QC materials and facilitate information exchange and communication on QC materials development, contribution, verification, distribution, and needs assessment. This CDC based Genetic Testing Quality Control Materials Program (GTQC) has designated a QC Materials Coordinator (QCMC) who will provide continuing support and coordination to improve QC material availability. The QCMC will 1) facilitate the identification, procurement, development, verification and distribution of needed QC materials; 2) facilitate exchange of QC-related information; and 3) explore collaborative efforts for ongoing needs monitoring and materials development. A GTQC website, available in March, 2005, will serve as an interactive communication tool to link users, providers, potential contributors, developers, and resources of QC materials and to inform the community about the availability of QC materials of clinical and public health interest.

The CDC convened three working meetings in 2003/04 to identify specific areas of need, develop recommendations for practical approaches and establish a sustainable, collaborative process to make QC materials available to the genetic testing community. These efforts will provide a sustainable, community-based process to make verified quality control materials available to the genetic testing community.

P1397. A quantitative approach to estimation and interpretation of pheno-caryotype relations in chromosome syndromes

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Application of principal component method of multifactorial analysis to real estimation of phenotypic variation in patients with partial autosomal aneuploidies is described. Data on 2345 respective patients from earlier elaborated database "CHRODYS" were used. Phenotypic correlations between the examined chromosome disorders were quantitatively estimated. By means of the applied analysis integral "portraits" of each kind of the examined autosomal aneuploidies could be revealed. The applied method differentiated up to 87 % of the aneuploidies in the sample by the first three principal components. Specific differences between the integral phenotypes of the aneuploidies were found. Unique diagnostically significant combinations of birth defects and microanomalies could be revealed for all the studied kinds of partial autosomal trisomies and/or monosomies distinguishing not only the chromosome number, but the chromosome arm, too. These combinations of traits were found in the great majority of patients with the given kind of chromosome disorder. High diagnostic value of the method in distinguishing the chromosome syndromes was shown. The obtained results may serve a basis, alongside with genetic, cytogenetic, and molecular genetic data, for the development and optimisation of diagnosing and monitoring of patients with chromosome disorders in genetic clinics.

P1398. Clinical benefit of Fabrazyme® (agalsidase beta) in Fabry disease: Results of the Phase 4 Study

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Fabry disease is an X-linked recessive disorder in which deficient activity of α -galactosidase A leads to pathogenic accumulation of neutral glycosphingolipids, primarily globotriaosylceramide. Enzyme replacement therapy (ERT) with Fabrazyme® has been shown to reduce accumulations in plasma and in lysosomes of renal, cardiac, and dermal cells. To assess clinical outcomes, a Phase 4 multinational, randomized, double-blind, placebo-controlled trial was conducted in Fabry patients who exhibited mild-to-moderate renal disease but had no prior ERT. Using 2:1 randomization, 51 patients

received biweekly infusions of 1 mg/kg Fabrazyme and 31 received placebo, with a median treatment period of 18.5 months. The primary efficacy endpoint was time to first clinically significant renal, cardiac, or cerebrovascular event. Results showed that Fabrazyme reduced the risk of any clinically significant event by 43% in the Intent-to-Treat population (including all randomized patients; n=82) and 46% in the Per-Protocol population (excluding major protocol violators; n=74). Baseline proteinuria was the most predictive factor for occurrence of renal ($p=0.001$) and all ($p=0.015$) clinically significant events, and, after adjusting for the baseline imbalance in proteinuria between the Fabrazyme and placebo groups, the risk reduction for Fabrazyme-treated patients increased to 53% ($p=0.058$) in the Intent-to-Treat and to 61% ($p=0.034$) in the Per-Protocol populations. Beneficial effects of Fabrazyme on serum creatinine levels and estimated GFR were more pronounced in patients who began ERT at less advanced stages of renal dysfunction. These findings indicate that Fabrazyme therapy exerts a positive impact on clinical outcomes and emphasize the benefit of early therapeutic intervention in Fabry patients.

P1399. Overall efficacy of agalsidase alfa in Fabry disease: results from FOS - the Fabry Outcome Survey

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Background: Fabry disease is an X-linked lysosomal storage disorder caused by deficient activity of the lysosomal enzyme α -galactosidase A. This multisystem progressive disorder leads to major organ failure and premature death. Enzyme replacement therapy (ERT) is now available, and FOS - the Fabry Outcome Survey - has been established to monitor the efficacy and safety of such therapy with agalsidase alfa.

Methods: FOS data were analysed to determine the effect of agalsidase alfa on the kidneys and heart and on health-related quality of life (HR-QoL).

Results: As of November 2004, 638 patients were enrolled in FOS from 11 European countries. Multivariate analysis of all the available creatinine data in FOS (n = 2800 measurements) showed a significant negative correlation between serum creatinine levels and the duration of agalsidase alfa treatment. Additionally, longitudinal data from 12 patients with a baseline GFR of 60-90 ml/minute revealed a significant decrease in renal function in the year before treatment, which was halted by 1 year of treatment with agalsidase alfa. Importantly, renal function was maintained in the second year of treatment. Cardiac structure (left ventricular mass and mean ventricular wall thickness) and function (midwall fractional shortening) were also significantly improved after 1 and 2 years of agalsidase alfa treatment. Sustained improvements in HR-QoL, assessed using the European Quality of Life Questionnaire (EQ-5D), were reported during 2 years of treatment.

Conclusion: Analysis of FOS data has confirmed the significant clinical benefits of ERT with agalsidase alfa in patients with Fabry disease.

P1400. Retrospective overview of 412 cases of hemangiomas

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Background: Hemangiomas are frequent during infancy. These are benign lesions which in some instances are difficult to classify either as tumors, hamartomas or malformations.

Objective: To identify clinical, genetic, and evolutive forms of hemangiomas as well as complications and therapy-related problems.

Lot of Study and Methods: We observed 412 cases of hemangiomas during 20 years (1984-2004) at the Clinical Children's Hospital Oradea. For each case, we made a clinical, genetic, therapeutic and evolutive evaluation.

Results: We classified our cases using three criteria: 1. clinical forms (tuberous, cavernous and angiomatic); 2. number of hemangiomas (single vs. multiples); 3. solitary hemangioma(s) vs. those associated with certain syndromes. We also divided cases by length of observation time: five, ten and more than ten years of follow-up. A large majority of hemangiomas are tuberous, single and solitary. The evolution was with partial or complete spontaneous resolution in the first five years in more than 90% of cases. Therapeutic intervention was required in only 9% of cases, and complications developed in less than 1% of cases

(ulcerations, infections, invasion of profound tissues).

Conclusions: Although prognosis is usually excellent in the vast majority of cases, certain patients with hemangiomas may develop severe complications and/or need serious medical interventions.

P1401. Inhibition of KRIT1 Expression in Endothelial Cells by RNA interference: A Model of Cerebral Cavernous Malformation

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Mutations in *KRIT1* gene (Krev1 Interaction Trapped gene 1) cause familial Cerebral Cavernous Malformation (CCM), an autosomal dominant disorder affecting primarily the central nervous system vasculature. CCM is characterized by a single layer of endothelium without normal intervening brain parenchyma or mature vessel wall elements. Despite the identification of *KRIT1* as the gene mutated in *CCM1*, the function of KRIT1 remains elusive. Recent studies aimed at understanding the function of the KRIT1 have demonstrated that it encodes a microtubule-associated protein and that it also interacts with ICAP-1 α . These data place KRIT1 at the cross-roads of signaling between the cytoskeleton and the ECM, potentially mediating the complex interaction between the extracellular and intracellular milieu. To gain a better understanding of a detailed description of the role of KRIT1 during cellular process, we inhibited KRIT1 expression in bovine aortic endothelial and HeLa cells by RNA interference. Using this technology, we explored the possibility that KRIT1 is involved in capillary tube formation. We found that suppression of KRIT1 expression resulted in decreased invasion, cell proliferation and increased apoptosis of endothelial cells. Interestingly, reduced KRIT1 expression also promoted decreased capillary tube formation in 3 dimensional culture system as one of the best model of in vitro angiogenic system. Based on these results, KRIT1 may take an active role in capillary formation. Furthermore, reducing KRIT1 expression in endothelial cells showed that utilization of siRNA oligonucleotides to reduce expression of KRIT1 may be a useful approach to understand the pathogenesis of cerebral cavernous malformation.

P1402. Reversal of gene expression profiles in the Phenylketonuria mouse by adeno-associated virus mediated gene therapy

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Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by a deficiency of phenylalanine hydroxylase (PAH). The accumulation of phenylalanine leads to severe mental and psychomotor retardation, and hypopigmentation. Phenylalanine restriction diet prevents irreversible damage if instituted from birth. Besides dietary therapy, several studies including gene therapy have been performed to develop methods that can efficiently remove excess phenylalanine. In this study, we identified transcription changes in effective treatment of PKU by the AAV-mediated delivery of a human PAH transgene. Oligonucleotide arrays were used to define gene expression profiles of *Pah^{enu2}* mice treated with and without AAV-mediated gene therapy. Therapeutic effectiveness was verified by enzyme activity and plasma phenylalanine level ($253.3 \pm 104.9 \mu\text{M}$) before microarray analysis. The gene expression profile, involved in cell cycle, immune reaction, cell adhesion, Ca^{++} transport, lipid metabolism, oxidative stress response and reproduction, was altered in the brain of untreated mice. In accordance with phenotype reversal, this altered gene expression pattern was reversed in the brain of *Pah^{enu2}* by AAV-mediated gene therapy. Actually, many similarities were observed in expression patterns between in treated *Pah^{enu2}* mice and wild type mice. This study may provide more comprehensive understanding about molecular level of PKU pathogenesis, results in possibility of better therapeutic approach.

P1403. Phase II Trial of Pirfenidone in Adult Patients with Neurofibromatosis Type 1 and Paraspinal and Plexiform Neurofibromas

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Neurofibromatosis Type 1 (NF1) is an autosomal dominant, progressive genetic disorder characterized by diverse clinical manifestations. Plexiform neurofibromas are major cause of morbidity in patients with NF1. The histopathology of these tumors is complex, with diverse cellular content and large amount of fibrous tissue, suggesting that events related to fibrogenesis might constitute a point of molecular vulnerability. Pirfenidone is a novel agent that exhibits potent anti-fibrotic activity in vitro and in vivo. We performed a pilot, open-label phase II trial of pirfenidone in adults with NF1 and paraspinal and plexiform neurofibromas to determine the effect on tumor size and to evaluate toxicities of this agent when taken on chronic schedule. Pirfenidone was administered orally in dose of 2400 mg/day for 24 months. Tumor size was monitored by a 3-D Magnetic Resonance Imaging (MRI). Seventeen patients completed 2 years of treatment. Six patients had symptomatic improvement of pain, three had improved neurologic function, while others remained stable without evidence of disease progression. One patient developed malignant peripheral nerve sheath tumor after completed 24 months of therapy. Seven patients were excluded from the study: three due to toxicity, two due to lack of compliance with follow-ups, and two due to persistent pain. Overall, pirfenidone was well tolerated. Lack of disease progression during 24-months of therapy in majority of patients is reassuring. However, given unknown natural history of these tumors, longer follow-up and larger population of treated patients are needed to determine the role of pirfenidone in therapy of NF1-related tumors.

P1404. Investigation of gene delivery with branched polylysine-based carriers

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Search for efficient carriers, which are able to deliver genes into eukaryotic cells and to provide their expression seems to be very important for gene therapy. Our study relates to two groups of branched polypeptide compounds (superbranched polylysines and dendritic polylysines). All vehicles were tested for their ability to bind and protect DNA from enzymatic degradation and for their transfection capacities in vitro. Modification of the basic carrier D1 (K₁₆-K₈-K₄-K₂-KA) with palmitoic acid residues resulted in increased transfection efficiency. Modification of D1 with arginine and amphipatic peptide fragment (compound DFP4) and increasing of generation number up to five (compound D5) did not lead to significant augmentation of transfection efficacy. Transfection efficacy of compound from the second group - superbranched polylysine - was very low (0.1%). The analysis of intracellular distribution of certain biotinilated DNA-carrier complexes suggests hampered DNA translocation from endosomes to cytosol as one of the main reasons for such low transfection capacity. Actually, the addition of endosomolytic agents - chloroquine or endosomolytic peptide JTS-1 - to the composition of polymer DNA-vehicles results in 5- to 50-folds increase in transfection efficiency. Thus modification of polypeptide structure provides a perspective tools in development of effective modular DNA vehicles.

P1405. The role of sterol 27-hydroxylase in atherosclerosis: bone marrow transplants in genetically modified mice

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Sterol 27-hydroxylase (CYP27A) is a mitochondrial enzyme involved in bile formation, however its extrahepatic expression has been implicated in novel mechanisms of cholesterol efflux. CYP27A

deficiency is found in cerebrotendinous xanthomatosis and leads to cholesterol and cholestanol accumulation in peripheral tissues resulting in neurological manifestations and possibly premature atherosclerosis. We overexpressed the human CYP27A in mice (CYP27^{tg}) and examined whether crossing these animals to apoE^{KO} mice would reduce atherosclerosis. These studies showed a non significant reduction in atherosclerosis in CYP27^{tg} on an apoE^{KO} background as compared to littermates without CYP27A overexpression.

In order to focus on the contribution of macrophage CYP27A to atherosclerosis we performed bone marrow transplants from CYP27^{tg}, C57BL/6J, and apoE^{KO} donors into apoE^{KO} recipients.

Apo E expression levels in bone marrow derived macrophages from CYP27^{tg} and C57BL/6J animals was sufficient to reduce hypercholesterolemia (190mg/dl and 200mg/dl compared to 1740mg/dl). Mean lesion area from cross sections showed reduced atherosclerosis in CYP27^{tg} bone marrow recipients as compared to C57BL/6J recipients (65µm² and 533 µm², respectively). No Sudanophilic aortic lesions were demonstrated in either CYP27^{tg} or C57BL/6J recipients. IL6 assays suggested a borderline shift towards an inflammatory pattern in CYP27^{tg} recipients.

Although apoE expression levels in macrophages from CYP27^{tg} and C67BL/6J donors were sufficient to reduce hypercholesterolemia and thus decrease atherosclerosis in apoE deficient mice, preliminary measurements suggest an atherosclerosis protective role for CYP27A. On the other hand, excess oxysterols in macrophages may promote IL6 secretion and contribute to inflammation in addition to their putative role in cholesterol efflux.

P1406. First cases of biotinidase deficiency in Russia

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Biotinidase deficiency (BD) is an autosomal recessive disorder of biotin recycling. The clinical features of untreated individuals commonly include seizures, hypotonia, alopecia, developmental delay, ataxia, breathing problems, hearing loss, optic atrophy and skin rash, ketolactic acidosis and organic aciduria. The disease can be treated successfully with biotin supplementation (between 5 and 20 mg per day). The most common mutation that causes profound BD is a deletion/insertion (G98:d7i3).

In the recent two years in our neurological department 3 patients have been detected with profound BD. The disease was confirmed by enzyme studies and mutation analyses in the BD gene. Two patients with zero activities were homozygous for the G98:d7i3 mutation and one was compound heterozygous for frequent mutations G₉₈d7i3/R538C. Clinical symptoms in our patients are similar: onset of disease on first months of life, combination of neurological symptoms (seizures, hypotonia, developmental delay, stridor, hearing loss) and cutaneous findings. All patients have demonstrated the dramatic improvements after two weeks of biotin (10mg/day) therapy. First the cutaneous symptoms and the seizures resolved, than signs of visual and hearing improvement began to be seen.

No clear genotype/phenotype correlation was detected. One of the patients homozygous for the frameshift mutation G98:d7i3 showed severe and the second one - mild clinical symptoms and better response for therapy.

These are first three cases of profound BD which were diagnosed and successfully treated in Russia.

P1407. Enzyme replacement therapy in Fabry disease : beneficial clinical effect in a patient with severely impaired kidney function.

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Fabry disease (FD, OMIM 301500) is an X-linked lysosomal storage disease caused by a deficiency of alpha-galactosidase. Two recent randomized trials have underlined the beneficial effect of enzyme replacement therapy (ERT) on biochemical parameters in patients with FD. However, although several case reports and uncontrolled studies have claimed clinical benefits, very few controlled data on clinical efficacy have been documented yet.

We report the case of a 46-year male patient whose severely impaired

renal function was stabilized with enzyme replacement therapy (ERT).

Results : Prior to enzyme replacement therapy, kidney function deteriorated rapidly from normal (serum creatinine : 125 $\mu\text{mol/L}$ in July 1999) to moderate (creatinine 275 $\mu\text{mol/L}$ in April 2002) and severe kidney insufficiency (serum creatinine 382 $\mu\text{mol/L}$ in December 2002) when ERT was initiated. The patient was subsequently followed during 26 months of ERT with alglucosidase beta (Fabrazyme®). The dose recommended by the manufacturer (1mg/kg of body weight every other week) was initially administered during 6 months. Serum creatinine reached a peak of 562 $\mu\text{mol/L}$ in June 2003. A doubling in the regimen (1 mg/kg weekly) was initiated for another 20 months in an attempt to rescue the kidney function. It is remarkable that although kidney function is still slowly deteriorating (creatinine value : 541 $\mu\text{mol/L}$ in January 2005) the patient is still not on dialysis.

To our knowledge this is the first observation of clinical efficacy of ERT in a Fabry patient with severely impaired renal function, potentially emphasizing the importance of the dose administered.