Abstracts

L01. Multiple Sulfatase Deficiency: Molecular defect and properties of the missing enzyme.

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Biochemie II, Georg August Universität Göttingen, Göttingen, Germany.

Based on purification and peptide sequencing of the missing enzyme (1) and complementation cloning using minicell mediated chromosome transfer (2) the gene defective in multiple sulfatase deficiency (MSD) was identified. The SUMF 1 gene encodes a protein that is located in the lumen of the endoplasmic reticulum, N-glycosylated and converts cysteine residue in the active site of newly synthesized sulfatases into Cα-formylglycine (FGly). This FGly-generating enzyme (FGE) is conserved from bacteria to man and contains three subdomains, separated by protease sensitive linker sequences. Two of the subdomains are linked by intramolecular disulfide bonds. The oxidation of cysteine to FGly is linked to the consumption of molecular oxygen. In the absence of FGE catalytically inactive sulfatase polypeptides are synthesized that contain cysteine instead of FGly , the biochemical hallmark of MSD.
Sequencing of 48 MSD-alleles of SUMF1 revealed in 44 alleles 21 different mutations. The spectrum of mutations comprises missense and nonsense mutations, deletions and insertions of single nucleotides and splice-donor site mutations. The effect of these mutations on the synthesis, stability, location, processing and activity of FGE is presently under investigation. In mammals a close paralog of SUMF1 1 gene is observed. This SUMF 2 gene encodes a protein that has a similar domain structure as FGE and is also located in the endoplasmic reticulum, but lacks FGE activity. The biological function of SUMF 2 remains to be determined.
1) T. Dierks et al, Cell 113 (2003) 435-444
2) M.P. Cosma, Cell 113 (2003) 445-456

L02. Biogenesis of mitochondria: Human diseases linked to protein transport, folding and degradation

***W. Neupert**;*

Adolf Butenandt-Institut Physiologische Chemie der Universität München, Munich, Germany.

Mitochondria are the „powerhouses“ of the cell. In these organelles energy present in oxidizable substrates is transduced into energy stored in ATP. Mitochondria have an own genome. In humans it encodes 13 proteins. However, the vast majority of proteins is encoded by nuclear genes, translated in the cytosol and then transported into the mitochondria. Proteins must then be folded and assembled to functional enzyme complexes. In addition, many proteolytic processes are required in mitochondria to maintain the steady state, such as processing of preproteins and proteolytic turnover of proteins. A number of protein complexes exist that mediate these various rather intricate reactions. These include:
(i) Preprotein translocases which facilitate the transfer of proteins and the insertion of proteins across and into the mitochondrial membranes, the TOM, TIM23, Tim22, and OXA complexes. (ii) A number of molecular chaperones and cochaperones, such as mitochondrial Hsp70s, Hsp60/10 and Mge1. (iii) The mitochondrial processing peptidase (MPP) which cleaves off the N-terminal targeting sequences, and the Lon and AAA-proteases which degrade matrix and membrane proteins in a regulated ATP-dependent manner.
The morphology of mitochondria is highly dynamic. Mitochondria are highly motile within the cell. Quite a number of genes are involved in these processes which are closely linked to the inheritance and maintenance of the mitochondrial genome.
In recent years, an increasing number of human diseases have been identified in which genes involved in the processes described above are involved. We will report on experiments aiming at understanding the biochemical basis of these diseases.

L03. New aspects of genetic mosaicism

***R. Happle**;*

Department of Dermatology, Philipp University of Marburg, Marburg, Germany.

The skin is especially suitable for the study of mosaicism because such phenotypes can be recognized most easily in the integument. There are two main types of mosaicism: epigenetic or genomic mosaicism. Recent research indicates that both X-linked and autosomal forms of epigenetic mosaicism can be caused by retrotransposon activity.
X-linked epigenetic mosaicism: Different patterns of lyonization include Blaschko lines (many syndromes), checkerboard pattern (X-linked hypertrichosis), and lateralization (CHILD syndrome).
Autosomal epigenetic mosaicism: This concept may explain the exceptional familial aggregation of pigmentary mosaicism that would visualize the action of a retrotransposon that is partly silencing and partly activating a pigment gene. Similar linear patterns are caused by retrotransposon activity in plants such as petunia or in animals such as mice or dogs.
Genomic mosaicism of lethal autosomal mutations: Some of these phenotypes have been elucidated at the molecular or cytogenetic level, but the genetic basis of Schimmelpenning syndrome and Proteus syndrome is still unclear. Phylloid hypomelanosis, a recently recognized neurocutaneous entity, is caused by mosaic trisomy 13.
Genomic mosaicism of nonlethal autosomal mutations: The hitherto prevailing theory that mosaic forms of autosomal dominant skin diseases always originate from a new mutation, is no longer valid. Today we distinguish two types of segmental manifestation. The type 1 reflects heterozygosity, whereas the type 2 results from allelic loss in a heterozygous embryo and shows pronounced lesions superimposed on the ordinary phenotype. This concept has now been proven at the molecular level in Hailey-Hailey disease.
Revertant mosaicism giving rise to unaffected skin areas in autosomal recessive cutaneous traits will certainly be recognized more often when clinicians are bearing this concept in mind. Such cases can be taken as examples of “natural gene therapy”.

L04. Regional differences in genetic testing and counselling in Europe - An overview

***S. Aymé**;*

INSERM, Paris, France.

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L05. Hereditary Breast/Ovarian Cancer risk: international comparison of the acceptability of Preventive strategies

***C. Julian-Reynier**;*

INSERM 379, Institut Paoli-Calmettes, Marseille, France.

The availability of genetic tests for BRCA gene mutations prompted cancer geneticists to give information about genetic risk and to assess many women with a personal or family history of breast or ovarian cancer to inform them of preventive measures. Previous results have shown international variations in women’s theoretical acceptability of the preventive strategies available. The highest level of acceptability was obtained for by mammographic screening, and chemoprevention was the secondly preferred option. Prophylactic oophorectomy and prophylactic mastectomy were thought to be acceptable before the age of 35 by a minority of the samples surveyed. A literature review will confront theoretical attitudes and actual behaviours of women at risk for HBOC observed in different countries. Several hypotheses to explain these results will be assumed among which the cultural component will be discussed more in depth.

L06. Variation in prenatal counselling in Europe: the example of Klinefelter

***T. M. Marteau**;*

Institute of Psychiatry, London, United Kingdom.

The organisation and delivery of prenatal counselling services varies across Europe. Focusing upon counselling for Klinefelter syndrome (KS), three questions are considered:
1. Does the content of prenatal counselling vary across Europe?
2. Is the variation in counselling associated with variation in termination rates?
3. To what extent is any variation to be reinforced or reduced?
Variation in counselling. Data from five European countries reveal

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that information reportedly given during prenatal counselling varies by specialty, country, and perceived quality of life: geneticists, German and UK health professionals and those who perceive a higher quality of life for people with KS report providing more positive information about the condition.
Variation in termination rates. The differences in reported counselling are reflected in termination rates observed in a cohort of 111 KS pregnancies from five Europe countries: women counselled by geneticists as opposed to other health care professionals are less likely to terminate an affected pregnancy (relative risk: 2.42, 95% CIs 1.14,5.92), with lowest termination rates seen in Germany.
Judging the variation. The variation in counselling and outcomes may reflect cultural differences in health professionals and general populations across Europe and hence is to be reinforced. Alternatively it may reflect care that militates against couples making informed choices about the outcomes of pregnancies affected by fetal abnormalities and hence is variation that should be reduced. The evidence needed to make this judgement will be discussed.

L07. Fact and Fiction Across Frontiers. Perceptions and Attitudes of families across Europe towards genetic testing and counselling

***L. Greene**;*

Climb, The National Information and Advice Centre for Metabolic Diseases, Crewe, United Kingdom.

As the Climb National information and Advice Centre for Metabolic Diseases, we are regularly approached by families requesting support from genetic services and more specific knowledge about the procedures for referral, genetic tests and the metabolic disease with which they are concerned.
Since metabolic diseases are rare, Climb frequently networks on an international basis for collaborative support with particular reference to NORD and EURORDIS .
Our experience at Climb suggests that some parents/patients have a greater anticipation of success and positive outcomes from genetic tests than is actually the case, while for others there is inherent fear and suspicion of the procedures involved.
Our experience also suggests that the specific needs of parents/patients such as knowing the potential benefits of genetic services, appropriate information, timely access to specialists and support from peer groups are not adequately addressed because of the lack of appropriately trained personnel and lack of resources, both human and financial.
Furthermore, Climb's experience suggests that the "expert" family can cause suspicion and resentment, inhibiting dialogue with practitioners where it should be enhanced. We also find that personality, levels of education and family and faith support systems play a part in increasing or diminishing resilience to the demands of handling the impact of genetic tests and counselling.
Common themes around expectations, attitudes, needs and perceptions therefore emerge.
Are any variations we experience based on geography, culture, health systems, attitudes or access? Climb is currently conducting a short survey in UK and Europe in collaboration with Eurordis to confirm or disprove this theory. What we must aim for is a more accessible, cohesive, sensitive and holistic service throughout Europe.

L08. Genome architecture, rearrangements, evolution and genomic disorders.

***J. R. Lupski**;*

Baylor College of Medicine, Houston, TX, United States.

The term "genomic disorder" refers to a disease that is caused by an alteration of the genome that results in complete loss, gain, or disruption of the structural integrity of a dosage sensitive gene(s). In most of the common chromosome deletion/duplication syndromes, the rearranged genomic segments are flanked by large (usually>10kb), highly homologous low copy repeat (LCR) structures that can act as recombination substrates. Recombination between non-allelic LCR copies, also known as non-allelic homologous recombination (NAHR), can result in deletion or duplication of the intervening segment. Recent findings suggest that other chromosomal rearrangements, including reciprocal, Robertsonian, and jumping translocations, inversions, isochromosomes and small marker chromosomes, may also involve susceptibility to rearrangements related to genome structure or architecture. In several cases, LCRs, AT-rich palindromes and pericentromeric repeats are located at such rearrangement breakpoints. Analysis of the products of recombination at the junctions of the rearrangements reveals both homologous recombination and non-homologous end joining (NHEJ) as causative mechanisms. Thus, a more global concept of genomic disorders emerges in which susceptibility to rearrangements occurs due to underlying complex genomic architecture. Interestingly, this architecture plays a role not only in disease etiology through constitutional rearrangements, but also in somatic rearrangement events associated with cancers and in primate genome evolution.

L09. Chromosome territory arrangements in the cell nucleus: probabilistic order and functional implications

***T. Cremer**;*

Department Biology II, Ludwig Maximilians University, Munich, Germany.

We present the first complete 3D maps of higher order chromosome territory (CT) arrangements in nuclei of postmitotic (G0) human diploid fibroblasts. Our data demonstrate that the radial CT order, i.e. the location of CTs in the nuclear centre or towards the nuclear periphery, is size dependent and highly non-random. In contrast, we note highly variable neighbourhoods of heterologous and homologous CTs. This pattern deviates strongly from the gene density correlated radial CT arrangements previously reported for lymphocyte nuclei and several other normal, human cell types. In all cell types studied so far the detected order is probabilistic and not rigidly deterministic. For a functional interpretation we propose the following hypothesis: In order to realize cell type specific gene expression and silencing patterns, transcribed, as well as non-transcribed, but potentially active genes must be positioned in euchromatic nuclear zones, permanently silent genes must be located in heterochromatic nuclear zones. In case of a differentiation dependent switch of gene expression, nuclear zoning must change accordingly either by a local change of the chromatin structure or by a switch of gene positions from a heterochromatic to an euchromatic nuclear zone or vice versa.
A. Bolzer, G. Kreth, I. Solovei, K. Saracoglu, C. Fauth, S. Müller, R. Eils, C. Cremer, M. R. Speicher, T. Cremer (2004) Complete 3D-maps of chromosome positions in human male fibroblast nuclei and prometaphase rosettes demonstrate a chromosome size dependent, probabilistic arrangement. Manuscript submitted.
M. Cremer, R. Zinner, S. Stein, H. Albiez, B. Wagler, C. Cremer, T. Cremer (2004) Three dimensional analysis of histone methylation patterns in normal and tumor cell nuclei. Eur. J. Histochem. 48, 11-23

L10. Williams syndrome

***L. Perez Jurado**;*

Unidad de Genética, Facultad de Ciencias de la Salud, Universitat Pompeu Fabra, Barcelona, Spain.

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L11. AAV vectors for the treatment of retinal and metabolic disorders

***A. Auricchio**;*

Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.

Recombinant vectors based on adeno-associated virus (AAV) serotypes are efficient tools to transfer genes to a variety of tissues, including retina, lung, liver and muscle. By exchanging the surface proteins among various AAV serotypes, vector mediated gene expression can be targeted to specific cell types in vivo influencing the site, time and levels of gene expression. We are currently testing AAV-mediated gene transfer of neurotrophic and anti-angiogenic proteins in animal models of retinal degenerations and neovascularization, resepectively. In addition, we are exploiting an "enhanced" gene therapy approach for lysosomal storage disease due to sulfatase deficiencies and an innovative system for

pharmacological regulation of insulin activity in type I diabetes.

L12. Enzyme replacement for Pompe disease

***A. van der Ploeg**;*

Department of Pediatrics, Sophia Children's Hospital, University Hospital Rotterdam, Rotterdam, Netherlands.

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L13. Management of hereditary dyslipidemia; from pharmacogenomics to gene therapy

***J. J. P. Kastelein**;*

Dept. of Vascular Medicine, Academic Medical Center, Amsterdam, Netherlands.

Hereditary dyslipidemia has provided unique insights into the molecular basis of human lipoprotein metabolism. For example, the elucidation of the low-density lipoprotein (LDL) pathway by Brown and Goldstein, rewarded by a Nobel prize in 1985, was only possible through the presence of patients with familial hypercholesterolemia (FH). In addition, this research lead to the development of HMG CoA reductase inhibitors, the most widely prescribed agents for the prevention of cardiovascular disease (CVD). All this knowledge has been instrumental in the understanding of atherogenesis and the role LDL particles have in this process.
On the other side of the spectrum, the HDL particle is seen as protective against cardiovascular disease. Using an extreme genetics approach, mutations have been discovered in a number of novel pathways leading to low HDL and a high risk for myocardial infarction. This, in consequence, has prompted research into the significance of HDL as a novel target for coronary artery disease (CAD) prevention. Similarly, defects in the triglyceride pathways have led to an understanding of its most pivotal enzyme, lipoprotein lipase. Patients lacking this protein are not treatable by medication but are excellent candidates for gene replacement therapy. So, in this presentation; gene discovery, mutation detection, pharmacogenomics and gene therapy of these intriguing hereditary disorders will be discussed.

LB1. *NIPBL*, encoding a homologue of fungal Scc2-type sister chromatid cohesion proteins and *Drosophila* Nipped-B, is mutated in Cornelia de Lange syndrome.

*E. T. Tonkin[1], T. J. Wang[1], S. Lisgo[1], M. J. Bamshad[2], **T. Strachan**[1];*

[1]Institute of Human Genetics, University of Newcastle, International Centre for Life, Newcastle upon Tyne, United Kingdom, [2]Department of Pediatrics and Department of Human Genetics University of Utah, Salt Lake City, UT, United States.

Cornelia de Lange syndrome (CdLS) is a multiple malformation disorder characterised by dysmorphic facial features, mental retardation, growth delay, and limb reduction defects. We have identified and characterised a novel gene, *NIPBL*, that is mutated in CdLS patients, and established its structure and that of rodent and zebrafish homologues, naming its protein product delangin. Vertebrate delangins exhibit significant homology to orthologues in flies, worms, plants and fungi, including Scc2-type sister chromatid cohesion proteins, and also *Drosophila Nipped-B*, which is known to regulate *Ultrabithorax* and *cut.* The pattern of embryonic *NIPBL* expression is generally consistent with the pathogenesis. Considering known Nipped-B interactions, we propose that perturbed delangin function causes inappropriate activation of *DLX* genes, thereby contributing to the proximo-distal limb patterning defects in CdLS. Very recently, *Nipped-B* has been shown to have a role in sister chromatid cohesion as well as in developmental gene regulation and we propose that vertebrate delangins also have a similar dual role that suggests possible parallels with the Roberts syndrome gene. The general role in sister chromatid cohesion could be satisfied by a basal level of expression but the role in target gene developmental regulation could be expected to require high expression in tissues where the target genes are active. Genome analyses typically reveal individual delangin/Nipped-B-like orthologues in diploid metazoan and plant genomes. The Scc2/Nipped-B/delangin family provides a model system for dissecting how a general chromosomal function has recently evolved to encompass an additional role in long-range developmental gene regulation.

LB2. A Molecular Pathogenesis for Transcription Factor Associated Poly-Alanine Tract Expansions

***A. N. Albrecht**[1], U. Kornak[1], A. Böddrich[2], K. Süring[1], R. Lurz[1], S. Stricker[1], E. Wanker[2], S. Mundlos[1];*

[1]Institute for Medical Genetics, Charité and Max-Planck Institute for Molecular Genetics, Berlin, Germany, [2]Max-Delbrück Center, Berlin, Germany.

Synpolydactyly is caused by expansions of a poly-alanine (Ala) tract in HOXD13 from 15 to 22-29 Ala. Subsequently, other disease causing Ala-expansions in transcription factors have been identified that cause developmental defects in humans and animals. Hoxd13 protein is normally localized in the nucleus, but concomitant with an increase of the Ala repeat above a certain treshhold (22 Ala) localization shifts from nuclear to cytoplasmic where the protein forms large amorphous aggregates. The rate of cytoplasmic aggregation vs nuclear localization of Hoxd13 correlates with the length of the repeat. Similar aggregates were observed for other expansions mutations in SOX3, RUNX2, and HOXA13 indicating a general mechanism. We analyzed the mouse mutant spdh harboring a +7 Ala expansion in Hoxd13 as an in vivo model and were able to show a reduction of mutant Hoxd13 and, in contrast to wt, a primarily cytoplasmic localization of the protein. Hoxd13 aggregate formation results in recruitment of heat shock proteins Hsp70 and Hsp40 and activation of the chaperone system by Geldanamycin leads to a reduction in aggregate formation. Recombinant mutant Hoxd13 protein forms aggregates in vitro demonstrating spontaneous misfolding of the protein. The length of the longest naturally occuring repeat in the human genome (20 Ala) is close to the threshhold for aggregation (22 Ala) observed in this study indicating an evolutionary restrain that, when exceeded, results in misfolding of the protein. Our results provide evidence that poly-Ala repeat expansions result in misfolding, degradation and cytoplasmic aggregation of the mutant proteins.

LB3. Germline mutations of the Ephrin-B1 gene cause Craniofrontonasal Syndrome

***I. Wieland**[1], S. Jakubiczka[1], P. Muschke[1], M. Cohen[2], H. Thiele[3], K. L. Gerlach[4], R. Adams[5], P. Wieacker[1];*

[1]Institut für Humangenetik, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany, [2]Medizinische Genetik im Kinderzentrum München, Munich, Germany, [3]Institut für Humangenetik, Martin-Luther-Universität Halle, Halle, Germany, [4]Klinik für Mund-, Kiefer- und Gesichtschirurgie, Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany, [5]Cancer Research UK London Research Institute, London, United Kingdom.

Craniofrontonasal syndrome (CFNS [MIM 304110]) is an X-linked craniofacial disorder with unusual manifestation pattern because affected females show multiple skeletal malformations like craniofacial asymmetry, hypertelorism as well as midline defects, whereas no or only mild abnormalities are manifest in male carriers. Recently, we have mapped a gene for CFNS in the pericentromeric region of the X chromosome including the *EFNB1* gene (MIM 300035) that encodes the ephrin-B1 ligand for Eph receptors. Since *Efnb1* mutant mice display a spectrum of malformations and unusual inheritance reminiscent of CFNS, we analysed the *EFNB1* gene in three CFNS families. In one family, a deletion of exons 2 to 5 was identified in an obligate carrier male, his mildly affected brother, and in the affected females. In two other families missense mutations in *EFNB1* were detected leading to amino acid exchanges P54L and T111I, respectively. Both mutations are located in multimerization and receptor interaction motifs within the ephrin-B1 extracellular domain. In all cases, mutations were consistently found in obligate male carriers, clinically affected males and affected heterozygous females. We conclude that mutations in *EFNB1* cause CFNS. This shows for the first time that mutations of the ephrin receptor/ephrin signal transduction system are associated with a Mendelian disorder and that ephrin signaling is involved in human skeletal development. Finally, we propose an explanation of the sex dependent manifestation of CFNS.

S01. Long QT syndrome what it means to patients

S. Priori;

Department of Molecular Cardiology, University of Pavia, Salvatore Maugeri Foundation, Pavia, Italy.

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S02. Genetic testing for Hypertrophic cardiomyopathy: how and what in clinical practice ?

P. Charron;

Département de Génétique, Hôpital Pitié-Salpêtrière, Paris, France.

Hypertrophic cardiomyopathy (HCM) is characterised by unexplained hypertrophy of the left ventricle and remains a leading cause of sudden death in teenagers and young adults, especially in athletes. HCM is an autosomal dominant disease caused by mutations in more than ten sarcomeric genes. Genetic testing is now available in selected high trained laboratories, raising new questions for the clinician in clinical practice.
Genetic testing for HCM can be discussed in different situations, such as diagnostic testing, prognostic stratification, predictive diagnosis, and prenatal diagnosis, all of which are associated with a variable degree of complexity and psychological implications.
Predictive testing is proposed to relatives of patients because it leads to the identification of relatives who will benefit from a specific cardiological follow-up, which might help decreasing the risk of sudden death. However, no medical treatment can be proposed at this stage. So, predictive genetic testing may result in adverse psychological consequences. The previous psychological burden because of uncertainty related to the genetic status could then be replaced by a new psychological burden about the near certainty of developing the disease later, including the risk of sudden death. To take into account the complex medical and psychological implications of this new approach, we developed a specific, multidisciplinary, and multiple step procedure, including a cardiologist, a geneticist, and a psychologist. The procedure and our preliminary experience will be described.

S03. Living with Marfan Syndrome: Perceptions of Cardiovascular Risk and Events

B. B. Biesecker[1], *F. Kong*[2], *R. Horne*[3], *K. Peters*[4];

[1]*National Human Genome Research Institute, Bethesda, MD, United States,* [2]*Westat, Inc, Rockville, MD, United States,* [3]*University of Brighton, Brighton, United Kingdom,* [4]*Pennsylvania State University, University Park, PA, United States.*

Motivated by an interest in the effects of living with the cardiovascular risks of Marfan syndrome, we conducted a cross-sectional survey of 174 adults. We assessed their perceptions of the condition and association to quality of life and adherence to medical recommendations. Respondents' quality of life was significantly decreased in the psychological/spiritual domain. Forty four percent reported clinical symptoms of depression independent of their use of channel blockade medications. Eighty three percent of respondents perceived Marfan syndrome as having had significant adverse consequences and this was correlated with pain (sore joints), depressive symptoms, and striae. A history of aortic dissection, sore joints and depressive symptoms were each negatively correlated with the view that Marfan syndrome is a curable/controllable condition. Eighty percent of respondents reported compliance with medication use and exercise restrictions. Yet they were more skeptical about the medications being essential for their health than reported for use of similar medications for other cardiovascular indications. Having to modify exercise was significantly correlated with an increased perception of Marfan syndrome as having negative consequences on respondents' lives. Overall, cardiovascular risks and events contributed to the perceptions of negative consequences of Marfan syndrome but less than daily disruptions such as modified exercise, joint pain and depressive symptoms.

S04. Using linkage disequilibrium patterns to map human complex trait loci

L. Cardon;

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

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S05. Human genetics of susceptibility to infectious agents: the example of mycobacterial infections

L. Abel;

Laboratory of Human Genetics of Infectious Diseases, University of Paris René Descartes, INSERM U550, Paris, France.

Although rarely pathogenic, poorly virulent mycobacteria, including live BCG vaccine and most environmental mycobacteria (EM), may cause a variety of clinical diseases, while *Mycobacterium tuberculosis* and *M. leprae* are more virulent, causing tuberculosis and leprosy, respectively. Remarkably, only a minority of individuals develop clinical disease, even if infected with virulent mycobacteria. The interindividual variability of clinical outcome is thought to result in part from variability in the human genes that control host defense. In this presentation, I will review the different methods and strategies that can be used to identify human genes controlling infectious diseases, and then the main findings concerning simple and complex inheritance of predisposition to mycobacterial diseases in humans. Rare patients with Mendelian disorders, affecting the IL-12/IFN-γ pathway, have been found to be vulnerable to BCG, a few EM, and *M. tuberculosis*. Substantial advances have been made in the genetic dissection of leprosy with the recent identification on chromosome 6q25 of two co-regulated genes (*PARK2* and *PACRG*) strongly associated with the risk of developing leprosy *per se* (all clinical subtypes). The studies carried out to date have been fruitful, initiating the genetic dissection of protective immunity against a variety of mycobacterial species in natural conditions of infection.

S06. The genetics of asthma and atopic dermatitis

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Text not available. Please check the website www.eshg.org/eshg2004 for submissions after the printing deadline.

S07. microRNA and RNAi machineries in mammalian cells

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In eukaryotes, dsRNA induces sequence-specific inhibition of gene expression at the level of mRNA degradation, known as RNAi. During RNAi, dsRNA is processed to ~21-bp siRNAs, which are incorporated into the RNA Induced Silencing Complex (RISC) to guide the cleavage of mRNA. miRNAs are ~21-nt RNA regulators interacting with 3'-UTRs of mRNAs and arresting translation by unknown mechanism. ~300 miRNAs are predicted to function in mammals. RNAi and miRNA pathways involve many proteins, either participating in siRNA and miRNA biogenesis, or functioning as components of RISC and miRNPs targeting mRNAs for degradation or translational repression.
Dicer is a multidomain nuclease responsible for biogenesis of siRNAs and miRNAs. Dicer domains include an RNA helicase/ATPase, DUF283 and PAZ and dsRBD domains, and two RNase III-like domains. Studies of human Dicer revealed its many interesting properties. In contrast to *Drosophila* and *C. elegans* enzymes, cleavage of dsRNA by the human Dicer is ATP-independent. The enzyme has a strong preference for cutting off siRNAs and miRNAs from substrate ends. Dicer is known to interact with Argonaute (Ago) proteins, established components of RISC. In collaboration with T. Hobman's group, we mapped domains responsible for the interaction. Dicer functions as a monomer, with its two RNase III domains associating together to form a pseudo-dimer, which contains a single processing center, generating siRNAs and miRNAs.
To get more insight into the mechanism of translational repression by miRNAs in HeLa cells, we uncoupled the miRNA generation/

assembly step from the direct effector step. We found that the miRNA-independent tethering of human Ago proteins to the 3'-UTR of mRNAs leads to the inhibition of translation by the miRNA-like mechanism. Hence, a primary function of miRNAs is to guide miRNP proteins to target mRNAs. Biochemical approaches to identify targets of miRNAs in mammalian cells will also be discussed.

S08. The Human Epigenome Project

***S. Beck**, on behalf of the Human Epigenome Consortium;*

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The Human Epigenome Project (HEP) is a public/private collaboration that aims to identify, catalogue and interpret genome-wide DNA methylation patterns of all human genes in all major tissues. Occurring naturally on cytosine bases at CpG sequences, DNA methylation is intimately involved in diverse biological processes and the aetiology of many diseases, particularly cancer. Differentially methylated cytosines give rise to distinct patterns thought to be specific for gene activity, tissue type and disease state. Such methylation variable positions (MVPs) are udeful epigenetic markers and promise to significantly advance our ability to understand and diagnose human disease.
As a prelude to the HEP, the Human Epigenome Consortium has recently completed a pilot study of the methylation patterns within the human Major Histocompatibility Complex (MHC), a region on chromosome 6 that is associated with more diseases than any other region in the human genome. This involved the development of an integrated pipeline for automated bisulphite treatment of DNA from minute tissue biopsies, gene-specific bisulphite PCR and sequencing of PCR amplicons. MVP discovery is aided by custom software and subsequent epigenotyping is carried out by MALDI mass spectrometry or microarray analysis. The pilot study entailed the analysis of over 100,000 CpG sites at over 200 MHC loci, including promoter and other relevant regions in multiple tissues and individuals. The generated data have been integrated with the human genome annotation using the ENSEMBL interface and are publicly available at http://www.epigenome.org.
Beck S, Olek A, Walter J. (1999) From genomics to epigenomics: a loftier view of life. Nat Biotechnol. 17:1144.
Novik KL, Nimmrich I, Genc B, Maier S, Piepenbrock C, Olek A, Beck S. (2002) Epigenomics: genome-wide study of methylation phenomena. Curr Issues Mol Biol. 4:111-128.
Denis C. (2003). Altered states. Nature 421:686-688.
Bradbury J. (2003) Epigenome Project - Up and Running. PLoS Biol. 1:E82.

S09. Imprinted Genes and Transposons: Epigenomic Targets Linking Fetal Nutrition with Adult Disease Susceptibility

***R. L. Jirtle**;*

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Human epidemiologic and animal data indicate that susceptibility to adult-onset chronic diseases such as cardiovascular disease, diabetes, obesity, and cancer is influenced by persistent adaptations to prenatal and early postnatal nutrition (Waterland and Jirtle, Nutrition 20:63-68,2004). Two potential epigenomic targets for early nutritional effects are imprinted genes and transposons. Imprinting is an epigenetic form of gene regulation that results in monoallelic parent-of-origin dependent gene expression (Murphy and Jirtle, BioEssays 25: 577-588, 2003). *IGF2* loss of imprinting is associated with an increased incidence of cancer (Cui *et al*., Science 299: 1753-1755,2003), and Beckwith-Weidemann syndrome in children conceived by *in vitro* fertilization (Gosden *et al*., Lancet 361:1975-1977,2003). We have now demonstrated in mice that early postnatal dietary methyl deficiency, and even exposure to a nutritionally complete synthetic diet, high in fat but low in fiber, causes biallelic expression of the oncogene, *Igf2*. Using viable yellow agouti (A^{vy}) mice, which harbor a retrotransposon in the *agouti* gene, we also showed that maternal dietary methyl donor supplementation during pregnancy alters coat color of the offspring via increased CpG methylation at the A^{vy} locus rather than by genetic mutation (Waterland and Jirtle, Mol. Cell. Biol. 23:5293-5300,2003). This epigenetic change also reduces the susceptibility of the offspring to obesity, diabetes, and cancer - a clear example of „nature via nurture." Thus, we are not only what we eat, but also potentially what our mother ate when we were in the womb. Our findings provide evidence that epigenetic alterations of transposons and imprinted genes can directly link environmental conditions during early development to the etiology of adult diseases. Further understanding these associations should make possible the development of early-life nutritional interventions or corrective therapies aimed at preventing chronic human diseases. (Supported by the NIH grants CA25951 and ES08823, AstraZeneca Pharmaceuticals and the Dannon Institute)

S10. Gene Expression networks in Breast Cancer

***A. Børresen-Dale**;*

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Breast cancers are heterogeneous and consist of several pathologic subtypes with different histological appearances of the malignant cells, different clinical presentations and outcomes, and the patients show a diverse range of responses to a given treatment. Furthermore, breast tumor tissue shows heterogeneity with respect to its microenvironment including specifically the types and numbers of infiltrating lymphocytes, adipocytes, stromal and endothelial cells. The cellular composition of tumors is a central determinant of both the biological and clinical features of an individual's disease.
By expression profiling we have identified five different subtypes of breast cancer by their global variation in gene expression patterns. The genes used for this classification were selected by their robust and steady expression between pairs of samples taken from the same tumor separated by weeks of chemotherapy treatment. Survival analyses showed significantly different outcome for patients belonging to the various subtypes. Differences in *TP53* mutation frequency between the subtypes indicated an important role for this gene in determining the gene expression pattern in the various tumors.
Cluster analyses of two published, independent data sets representing different patient cohorts, uncovered some of the same breast cancer subtypes. By including a group of tumors from *BRCA1* carriers in the analysis we found that this genotype predisposes to the subtype identified as basal like.
Our results strongly support the idea that many of these breast tumor subtypes represent biologically distinct disease entities, and that both the patients genotype and the tumor genotype have a strong influence on the expression pattern developed in a given tumor.
Nature: 406, 747, 2000, PNAS: 98, 10869, 2001, Molecular Intervention, 2 no. 2 , 101, 2002, PNAS: 99: 12963-12968, 2002, PNAS: 100: 8418-8423, 2003. Mol.Biol.Cell. In press 2004 (Zhao H., Langerød, A. et al).

S11. Dependence receptors : cell death inducers and conditional tumor suppressors

***P. Mehlen**;*

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Dependence receptors are receptors that display two totally different signal transductions depending on ligand availability (1-5). Indeed, if in the presence of ligand, these receptors transduce a positive signal leading to differentiation/proliferation/migration, in the absence of ligand these receptors are not inactive but induce an active process of cell death. Thus, such receptors create cellular states of dependence on their respective ligands by inducing apoptosis when unoccupied by ligand. The DCC (Deleted in Colorectal Cancer) gene, a candidate tumor suppressor gene, encodes a receptor for netrin-1, a laminin-related molecule involved in axon guidance. In 1998, we reported that DCC is a dependence receptor since its expression induces apoptosis, a pro-apoptotic activity blocked in the presence of netrin-1 (1). A similar behavior was also found for another netrin-1 receptor, UNC5H, hence suggesting that netrin-1 is not only a chemotropic factor but a survival factor via its receptor DCC and UNC5H (3). Thus, these dependence receptors play the role of a switch driving either a positive signal of differentiation (4,5) or a negative signal of cell death. Remarkably both DCC and UNC5H expression appear drastically inhibited in numerous carcinomas

including colorectal tumors (8). Here we will present evidence here that UNC5H and DCC may be considered as tumor suppressors that limit tumor growth out of netrin-1 availability by inducing apoptosis.

S12. Methylation in cancer

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S13. Photoreceptor biology in drosophila

***C. Desplan**;*

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S14. The role of A2-E in Macular Degeneration

***F. G. Holz**;*

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Age-related macular degeneration (AMD) is now the leading cause of blindness in the industrialized world. Several lines of evidence suggest that ageing changes of the retinal pigment epithelium (RPE), a monolayer between the choroid and the neurosensory retina with essential functions for normal vision, play a key role in the pathogenesis of the disease. In postmitotic RPE cells autofluorescent lipofuscin granules accumulate with age in the lysosomal compartment in association with AMD as a complex, multifactorial disease as well as in monogenetic macular dystrophies including Best's and Stargardt's disease. Lipofuscin accumulation as a common downstream pathogenetic pathway is mainly a byproduct of constant phagocytosis of membranous discs shed from distal photoreceptor outer segments and posttranslational modifications by oxidative damage. We were able to show that the major retinoid compound of RPE lipofuscin, N-retinylidene-N-retinylethanolamine (A2-E), severely affects lysosomal functions of RPE. This effect is mediated by the inhibitory action of A2-E on the ATP-driven lysosomal proton pump. The resulting pH shift in the lysosomal compartment causes an inhibition of lysosomal hydrolytic enzymes, triggering severe lysosomal dysfunction, which ultimately results in RPE cell dysfunction and cell damage. Such sequence of events is likely to contribute to the biogenesis of drusen with release of incompletely degraded material into the extracellular matrix of Bruch's membrane at the basal cell side. Furthermore, A2-E has been shown to possess phototoxic and detergent properties upon reaching critical levels. Several animal models have been developed that display accelerated RPE lipofuscin/A2-E accumulation including abcr(-/-) knockout and Ccl-2-deficient mice. These will be helpful to identify potential targets for intervention. The pathophysiological role of lipofuscin in vivo is underscored by recent fundus autofluorescence findings with the use of confocal scanning laser ophthalmoscopy which showed that excessive lipofuscin levels precede cell death in outer reinal layers with subsequent loss of visual function.

S15. From epithelial cell polarity to retinal degeneration: lessons from Drosophila

***E. Knust**;*

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S16. Kremens are novel Dickkopf receptors that regulate Wnt/β-catenin signalling

***C. Niehrs**[1], B. Mao[1], W. Wu[1], G. Davidson[1], J. Marhold[2], M. Li[2], M. Mechler[2], H. Delius[3], A. Glinka[1];*

[1]Division of Molecular Embryology, Deutsches Krebsforschungszentrum, Heidelberg, Germany, [2]Division of Developmental Genetics, Deutsches Krebsforschungszentrum, Heidelberg, Germany, [3]Division of Applied Tumorvirology, Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Canonical Wnt signalling via the β-catenin pathway is transduced by two receptor families. Frizzleds and Lipoprotein receptor related proteins 5 and -6 (LRP5/6) bind Wnts and transmit their signal by stabilizing intracellular β-catenin. Wnt/β-catenin signalling is inhibited by the secreted protein Dickkopf1 (Dkk1), member of a multigene family, which acts as head inducer in the Spemann organizer of amphibian embryos. Recently, Dkk1 was shown to inhibit Wnt signalling by a novel mode of action, by binding to and antagonizing LRP5/6. We now describe the transmembrane proteins Kremen1 and -2 as novel high affinity Dkk1 receptors, which functionally cooperate with Dkk1 to block Wnt/β-catenin signalling. Kremen2 forms a ternary complex with Dkk1 and LRP6 and induces rapid endocytosis and removal of the Wnt receptor LRP6 from the plasma membrane. The results indicate that Kremens are components of a membrane complex modulating canonical Wnt signalling through LRP6 in vertebrates.

S17. Viral vectors as tool to create animal models of CNS disorders

***N. Deglon**[1,2];*

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The identification of mutations implicated in familial forms of CNS diseases has led to the production of transgenic mice. Recently, the injection in specific brain areas of viral vectors encoding disease-causing genes has been proposed as alternative strategy to create genetic models. This strategy holds various advantages. Viral vectors are versatile, flexible tools to perform *in vivo* studies. Multiple genetic models can be created in a short period of time. High transduction efficiencies, as well as robust and sustained transgene expression lead to the appearance of severe functional and behavioral abnormalities. Targeted injections in different brain areas can be used to investigate the specificity of the neuropathology and eliminate side effects associated with a widespread transgene overexpresssion. Finally, models can be established in different mammalian species, thereby providing an opportunity to assess complex behavioral changes and perform longitudinal follow-up by imaging in primates. The proof of principle of the approach was established with lentiviral vectors encoding the gene causing Huntington's disease. Viruses expressing the huntingtin (htt) protein with 19 (wild type) or 82 (mutated) CAG repeats were injected in striatum of adult rats. Lentiviral-mediated delivery of htt-82Q but not htt-19Q induced a progressive pathology characterized by the sequential appearance of ubiquitinated aggregates, followed by neuronal dysfunction, astrogliosis, leading finally to a robust and selective degeneration of striatal neurons. Recently, we have scale-up the approach in primates and assessed whether behavioral deficits were associated with a striatal pathology. The unilateral overexpression of htt-82Q in the dorso-lateral putamen was associated with an increase in locomotor activity, an ipsilateral turning under apomorphine and the occurrence of typical choreic-like, dystonic movements. These data demonstrate that lentiviral-mediated expression of mutated htt provides a robust *in vivo* genetic model that will facilitate future studies on the pathogenesis of cell death and experimental therapeutics for HD.

S18. Combining HT-RNAi and high-content assays in human cells and model organisms for target discovery and validation

***B. Sönnichsen**;*

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We have established an automated platform for genome wide HT-RNAi screening in cultured human and Drosophila cells, as well as C. elegans. Having completed several such projects using either very high content readout assays (e.g. time-lapse microscopy), or more focused, low content readouts (e.g. plate reader assays), case studies using both approaches will be discussed to illustrate the method's overall potential. First, a high content screen for new cell division genes was completed using systematic RNAi covering 99.2% of all predicted C. elegans genes. Double stranded RNAs (dsRNAs) were microinjected into adult worms and resulting phenotypes were recorded 24hrs later during the first two divisions of F1 embryos using timelapse DIC microscopy. Particular effort was put into standardizing

analysis of this high content screen. A second, more focused RNAi screen has covered 86% of the Drosophila genome, implicating 19 genes in a novel cellular antiviral response pathway. Here, S2 cells were soaked in dsRNAs using 384 well plates followed by automated microscopy and image analysis. Third, systematic siRNA-based screens in human cells have been carried out for defined panels of genes. Results will be discussed at the meeting.

S19. Pharmacogenetics

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S20. Genetics of the antidepressant response

***E. Binder**, F. Holsboer;*

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Depression is a very common medical disorder, with lifetime prevalences of up to 14%, thus affecting millions worldwide. While antidepressants are the most effective treatment for depressive disorders, there still is substantial need for improvement of therapy. In the lack of objective criteria for the choice of the optimal antidepressant treatment depressed patients are treated on a trial and error basis, resulting in a failure rate of a single treatment attempt in up to 30-40%. Pharmacogenetic approaches may open up an avenue for an individualized and improved antidepressant therapy. At the Max-Planck Institute of Psychiatry, we have investigated about 70 candidate genes from systems potentially involved in the mechanism of action of antidepressant drugs for their association with response to antidepressant treatment. So far over 400 depressed in-patients have been recruited at our Institute within the Munich Antidepressant Response Signature (MARS) project and characterized in weekly intervals for their response to antidepressant treatment using the Hamilton Depression Rating Scale. We found a strong association of single nucleotide polymorphisms (SNPs) in FKBP5, a glucocorticoid receptor regulating co-chaperone of hsp90 with response to antidepressant drugs ($p = 5.5 \times 10\text{-}6$). These SNPs were also associated with a higher expression of FKBP5 protein in lymphocytes. We also found an association of a rare functional SNP in the glucocorticoid receptor itself that leads to a partial glucocorticoid receptor resistance with response to antidepressant treatment ($p = 0.0049$). Our results, as well as those from other research groups appear to be promising for the future development of an individualized antidepressant pharmacotherapy.

S21. The polypill

***M. Law**;*

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S22. The complex nature of constitutional *de novo* apparently balanced translocations in patients presenting with abnormal phenotypes

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With the publication of the complete human genome sequence, a complete set of mapped and sequenced DNA clones are now available which can be utilised as a valuable resource for molecular cytogenetics. In particular, this resource has facilitated the production of high resolution DNA microarrays (Fiegler et al. 2003a) which most commonly have been used to detect imbalances in whole genomic DNA using comparative genomic hybridisation (CGH). Recently, we have reported a novel technique termed array painting, which is a combination of technologies involving the hybridisation of flow sorted chromosome material onto DNA microarrays (Fiegler et al. 2003b). We are using array painting to map rapidly the breakpoints of aberrant chromosomes have used array CGH and array painting to analyze constitutional *de novo* apparently balanced translocations in patients presenting with abnormal phenotypes. The use of microarrays to compliment cytogenetic analysis allows efficient screening and characterization of the whole genome for imbalance as well as rapid identification of breakpoint regions. This greatly facilitates finer mapping by FISH and other methods. We have identified additional complexity or genome imbalance in 60% of patients analyzed without knowledge of their phenotypes and these results sub-divided the patients into three groups: (1) translocation breakpoints which appeared simple and balanced at the resolution used; (2) complex rearrangements, some including deletions, inversions and insertions at or near one or both breakpoints; (3) cases in which the translocations appeared balanced but were found by microarray analysis to have a previously unrecognized microdeletion or duplication not associated with the translocation. These results, if generally confirmed in the study of further patients, will have a significant impact on current diagnostic investigations of this type and provide an argument for the more widespread adoption of microarray analysis or other high resolution genome wide screens for chromosome imbalance and rearrangement.

S23. Molecular analysis of breakpoint sequences in non-recurring balanced translocations

***R. Giorda**[1], U. Pozzoli[1], M. Bonaglia[1], S. Giglio[2,3], G. Gimelli[4], G. Gregato[2], T. Pramparo[2], M. Sironi[1], C. Baschirotto[1], O. Zuffardi[2,3];*

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Gross rearrangements (deletions, duplications, translocations) are important causes of cancer and inherited disease, and for this reason they have been the subject of intense study. These rearrangements are nonrandomly distributed in the human genome. One of the reasons is of course a sampling bias: in cancer, somatic rearrangements must confer a growth advantage in the affected cells, while germ-line rearrangements are usually detected only when they cause a clinical phenotype, either in the subject or in his/her descendants.

A second reason is the presence of recombination "hotspots" that have often been associated with some recurrent and non-recurrent rearrangements; these DNA sequences promote either homologous unequal recombination or non-homologous recombination. Homologous unequal recombination is mediated by paralogous regions on homologous or non-homologous chromosomes, and generates a variety of deletions, duplications and translocations. Recurrent non-homologous recombination seems to be related to the presence of specific, potentially unstable sequences, often due to the presence of clustered repeated sequences named Low Copy Repeats (LCR); these recombinations yield breakpoints containing short deletions and insertions.

Fewer efforts have been devoted to the analysis of breakpoints of non-recurrent translocations, because they occur less frequently, they are sometimes harder to discover and until recently their molecular characterization was technically more demanding.

We have analyzed the breakpoints of 26 germ line, non-recurrent, balanced autosomal translocations, 10 of them characterized in our laboratory, 16 obtained from published reports. The oligonucleotide composition, presence of recombination-associated motifs and secondary structure of each breakpoint were compared and matched to a set of randomly generated reference sequences.The results are consistent with a role for non-homologous recombination in the genesis of non-recurrent autosomal translocations, while regional sequence structure may be involved in generating some of the breakpoints.

S24. Translocation breakpoints and disease genes

***V. Kalscheuer**;*

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Disease-associated balanced chromosomal rearrangements (DBCRs) that inactivate specific genes form visible bridges between genotypes and phenotypes. We and others have shown that the systematic study of DBCRs is a very powerful strategy for identifying genetic changes underlying human disease. To date

we have analysed more than 150 patients with DBCRs that were available through close collaboration with the Mendelian Cytogenetic Network (N. Tommerup, Cophenhagen) and interactions with clinical geneticists worldwide. So far, we have identified more than 35 genes disrupted by the chromosomal rearrangement in patients with mental retardation or related disorders. These genes code for a wide variety of functionally interesting proteins, including regulators of Rho GTPases, kinases, phosphatases, transcriptional regulators, synapse-associated proteins, axon guidance molecules, transporters and factors involved in protein degradation. Thus, DBCRs are a rich source of genes that play a role in the development and function of the human brain and other organs, and their ongoing functional characterization should shed more light on the role of these genes in the etiology and pathogenesis of inherited disorders. In a small percentage of cases breakpoint fine-mapping and cloning revealed the presence of a small deletion or complex rearrangement. In other cases the clinical phenotypes can be explained by position effects or by the disruption of long-range regulatory elements. More recently, we have started to extend our systematic study of DBCRs to rearrangements with complex and late-onset diseases.

S25. Affinity Proteomics to Explore the Human Genome

***M. Uhlen**;*

Royal Institute of Technology (KTH), Stockholm, Sweden.

A systematic approach to convert genomics data into biological knowledge based on protein profiling is described. The strategy relies on a high-throughput method for the recombinant production of non-homologous regions of the proteome selected by whole genome bioinformatics. Such protein fragments are individually used to generate and enrich mono-specific antibodies for systematic analysis of protein profiles (expression and localization) in different human organs using tissue arrays The results suggest that this Affinity Proteomics strategy can be used to produce a proteome atlas, describing distribution and expression of proteins in normal tissues as well as in common cancers and other forms of diseased tissues. The implications for the development of protein arrays will be discussed.
Agaton et al (2003) Affinity proteomics for systematic protein profiling of chromosome 21 gene products in human tissues. Mol Cell Proteomics 2(6): p. 405-14.

S26. Function prediction and protein networks

***P. Bork**;*

EMBL Heidelberg, Heidelberg, Germany.

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S27. Genome-wide Cell based RNAi Screens in Drosophila

***M. Boutros**;*

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A crucial aim upon completion of whole genome sequences is the functional analysis of predicted gene products. Drosophila is one of the best-studied genetic model systems and has been instrumental to the identification of conserved pathway components with important roles from flies to humans.
We have developed a RNAi library containing 21306 fragments against almost every predicted gene in the Drosophila genome. Treatment of cultured Drosophila cells with dsRNA leads to the efficient depletion of the corresponding transcript and the generation of specific and penetrant phenotypes.
We have here applied a high-throughput RNAi screen in macrophage-like cells to characterize the function of nearly all predicted Drosophila gene in cell growth and viability. We found several hundred dsRNAs that identified essential genes, among which 80% lacked mutant alleles in vivo. Quantitative analysis showed that mutant phenotypes allowed classification of phenotypes into distinct classes, ranging from growth inhibition to programmed cell death.
We now apply the combination of a genome-wide RNAi library and massively parallel phenotyping to systematically dissect a variety of cellular pathways. The genome-wide RNAi library is adaptable for screening for many different cellular pathways and processes, which should ultimately facilitate the understanding of complex cellular networks.
Boutros *et al.* (2004). Genome-wide RNAi Analysis of Growth and Viability in Drosophila Cells. Science 306: 832-835

S28. Genetic architecture of Hsp90-buffered traits

***S. Rutherford**, K. F. Gorman, R. Howsmon, J. Biava, C. Carey, A. Aragaki, C. Kooperberg;*

Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

Under low Hsp90 previously hidden genetic and epigenetic variation for rare morphological abnormalities is revealed in Drosophila and other organisms, inspiring the 'Hsp90 capacitor' hypothesis for morphological evolution. To understand Hsp90 genetic buffering, we used QTL and deletion mapping to find genes responsible for a common, Hsp90-dependent *deformed eye* trait (*dfe*). Three replicate fly lines started from a single deformed male and four related, but normal, females were selected over many generations, increasing the probability of *dfe* until nearly every fly in each line was abnormal. Like human disease, *dfe* is a complex trait; a threshold sensitive to many genetic and environmental factors determines whether *dfe* is expressed. Outside the threshold, phenotype is insensitive to genotype and mapping power is poor. Over a small range of liability, risk increases sharply, and phenotype (disease or deformity) is sensitive to even minor genetic effects. To map *dfe* near its threshold, we placed small random intervals from an inbred wild background (normal eyes) into each high line to make isogenic recombinant lines (IRLs). IRL penetrance was high unless recombinant intervals contained dominant wild-type alleles of *dfe* genes that suppressed its penetrance. This design powerfully identified several shared and unique QTL across the replicate mapping experiments and will facilitate their unambiguous identification. Many QTL corresponded to one or more genes or regions found independently by mapping non-complementing deletions. Genes affecting Hsp90 pathways accumulate as neutral variants in normal populations. The many Hsp90-buffered genes contributing interchangeably to *dfe* liability from variation in just a few flies suggest Hsp90-buffered variants affecting eye development, like genes accounting for a large proportion of risk for human disease, are common but usually unexpressed. Many Hsp90 pathways are misregulated in cancer. Cloning Hsp90 buffered variation from normal fly populations will identify new candidate genes for cancer and other complex diseases.

S29. Pituitary development

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S30. Cholesterol Biosynthetic Disorders - What Can We Learn from Mouse Models?

***G. E. Herman**;*

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Since 1998, five human disorders involving enzyme defects in post-squalene cholesterol biosynthesis have been identified - desmosterolosis, X-linked dominant chondrodysplasia punctata, CHILD syndrome, lathosterolosis, and hydrops-ectopic calcification-moth-eaten skeletal dysplasia. They join the most common disorder, Smith-Lemli-Opitz syndrome, whose underlying defect was identified in 1993. All are associated with major developmental malformations that are unusual for metabolic disorders. In 1999, we determined that the X-linked, male lethal mouse mutation bare patches (*Bpa)* results from mutations in a novel 3β-hydroxysteroid dehydrogenase (*Nsdhl*) involved in removal of C-4 methyl groups in a late step of cholesterol biosynthesis. We are employing this mouse model to examine the pathogenesis of cholesterol biosynthesis disorders. The NSDHL protein is localized on the surface of lipid droplets, in addition to ER membranes, suggesting that it may play a role in intracellular cholesterol trafficking. Affected male embryos die in mid-gestation between E9.5-E12.5, depending on the allele. Cholesterol and total sterol levels are normal in affected males at

the time of death, suggesting that the fetal pathology is not caused by a lack of cholesterol. In addition, while there are no consistent anomalies noted in affected embryos, the associated placentas are always small and demonstrate a thinner, disorganized labyrinth and fewer fetal vessels. Since most of the extraembryonic lineages in the mouse demonstrate non-random X-inactivation, we hypothesize that cells from extraembryonic mesoderm that demonstrate random X-inactivation and contribute to the labyrinth are responsible for the severe, male lethal phenotype. These mesodermally derived cells contribute to the endothelial lining of fetal vessels in the labyrinth, consistent with the abnormalities we have identified. We are currently using techniques such as in situ hybridization, generation of transgenic mice, and microarray expression analysis to further examine the male lethality and to help provide clues about the pathogenesis of the human cholesterol biosynthetic disorders.

C01. Phenylbutyrate increases *SMN* gene expression and motor function in spinal muscular atrophy patients

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease, characterized by degeneration of the anterior horn cells of the spinal cord. SMA is caused by insufficient levels of survival motor neuron (SMN) protein. No cure for SMA is available at present. Increasing SMN expression could be of considerable therapeutic importance.

Recently, we found that sodium 4-phenylbutyrate (PB), a well tolerated FDA approved drug, enhances *SMN* gene expression *in vitro*. To assess whether PB is effective to increase SMN expression also in vivo we have administered oral PB (*tri*Butyrate®, Fyrklövern Scandinavia AB) to 6 patients for 7 days and determined leukocyte SMN mRNA levels by real-time PCR. We observed for all patients a significant increase in relative SMN full length transcript levels in one or more blood samples obtained during PB administration compared to baseline ($p<0.005$). To evaluate tolerability and clinical efficacy of PB we have performed a pilot study including 10 SMA type II patients to whom oral *tri*Butyrate® was administered for 9 weeks using an intermittent schedule (7 days on and 7 days off). Changes in motor function were evaluated using the Hammersmith functional motor scale. We found a significant increase in the scores of the Hammersmith functional scale at both 3 weeks ($p<0.012$) and 9 weeks assessments ($p<0.004$) compared to baseline.

Our results indicate that PB might be beneficial to SMA patients without producing any major side effect. Larger prospective randomised placebo controlled trials are needed to confirm these preliminary findings.

C02. Pilot study of in-vivo effects of valproic acid on *SMN* gene expression in SMA carriers

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Spinal muscular atrophy (SMA) is a frequent inherited motoneuron disease caused by homozygous mutation of the survival motoneuron gene 1 (*SMN1*). An almost identical gene copy, *SMN2*, fails to compensate for the loss of *SMN1* due to a silent mutation leading to skipping of exon 7 in the majority of *SMN2* transcripts. These encode a truncated, unstable protein that is not functionable.

Recently, we demonstrated that therapeutic doses of valproic acid (VPA), a drug successfully used in epilepsy treatment, increase full-length (FL) *SMN2* mRNA and protein levels in SMA fibroblasts by stimulating transcription and promoting exon 7 inclusion. Based on these results, our group started a pilot trial with VPA in 17 SMA carriers, each of them carrying 1 *SMN1* copy and 1-3 *SMN2* copies. Due to elevated liver values, 7 probands had to be excluded. The first 3 blood samples of the remaining probands were taken without medication. Then, treatment was started by administering 300 mg of VPA/day, gradually increasing the dose up to 1200-1800 mg/day to achieve a blood level of 70-100 µg VPA/ml (common in epilepsy therapy). Blood levels of FL and truncated *SMN* mRNA were determined applying real-time quantitative PCR, at the same time evaluating the suitability of several housekeeping genes for use as stably expressed standard. SMN protein levels in peripheral blood mononuclear cells (PBMCs) were analyzed by flow cytometry. Data will be presented to characterize the in-vivo effect of VPA on *SMN* gene expression in PBMCs as potential parameter for monitoring further clinical studies.

C03. In vivo enhancing effect of *SUMF1*, the Multiple Sulfatase Deficiency gene, on sulfatase activities

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Human sulfatases cleave sulfate esters from a wide range of substrates and deficiencies of these activities cause at least eight human monogenic diseases. We have recently identified the gene for Multiple Sulfatase Deficiency (MSD) named *SUMF1* (SUlfatase Modifying Factor 1) which is responsible for converting a cysteine into formylglycine at the active site of all human sulfatases. Co-transfection studies in cell lines demonstrated SUMF1 enhancing effect on sulfatase activities. To date, gene therapy protocols for sulfatase deficiencies required unexpectedly high levels of exogenous sulfatases to correct the metabolic defect. We hypothesized that simultaneous delivery of *SUMF1* and sulfatase genes results in a more effective treatment of diseases due to sulfatase deficiencies. We have delivered mono and bicistronic AAV vectors encoding the human *IDS and ARSB* genes with and without *SUMF1* to murine liver, lung, muscle, CNS and HSC. Our preliminary results in transduced muscle show SUMF1 enhancing effect on ARSB activity which is secreted and uptaken by uninjected tissues, supporting the validity of this novel approach for gene therapy of sulfatase deficiencies.

C04. Functional overlap between ABCD1 (ALD) and ABCD2 (ALDR) transporters: a therapeutic target for X-linked adrenoleukodystrophy

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X-linked adrenoleukodystrophy (X-ALD), a devastating, neurodegenerative disease, is the most common genetic cause of leukodystrophy and Addison's disease, and the most common peroxisomal disorder (1 in 17:000 males). X-ALD is the consequence of mutations in the ABCD1 (ALD) gene that encodes a peroxisomal ABC transporter. Its closest homologue, ABCD2 (ALDR, 88% homology to ALD) is able to rescue the biochemical defect (very-long-chain fatty acid accumulation) when overexpressed in vitro. We have recently uncovered a late-onset neurodegenerative phenotype in the ageing ALD- mice, that resembles adrenomyeloneuropathy in patients, or late-onset ALD. Using this model, we have addressed the functional redundancy of both transporters in vivo, by generating in one hand, mice overexpressing ALDR and in the other hand, knock-out mice for ALDR. Overexpression of the ALDR gene can indeed prevent the clinical and histopathological phenotype, while the phenotype of mice deficient for both ALD and ALDR is more severe and of earlier onset, and in particular these mice present signs of inflammatory reaction. Inflammatory reaction in brain is a hallmark of the severe childhood form of X-linked adrenoleukodystrophy, but had not been observed in the single ALD- mouse. We thus demonstrate that the ALDR gene is a valid therapeutic target for adrenoleukodystrophy. As this gene is regulated by Ppara and Srebp transcription factors, and upregulated by thyroid hormone and inhibitors of histone deacetylases such as 4-phenylbutyrate,

pharmacological upregulation of ALDR gene expression appears a feasible approach towards treatment of adrenoleukodystrophy.

C05. Paradoxical NSD1 mutations in Beckwith-Wiedemann syndrome and 11p15 anomalies in Sotos syndrome

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Sotos syndrome is an overgrowth syndrome characterized by pre- and postnatal overgrowth, macrocephaly, advanced bone age, typical facial gestalt and variable degree of mental retardation. Aberrations of the NSD1 gene account for largely 80% of Sotos syndrome, while the disease mechanism of other cases remains unknown.
Macrosomia, macroglossia, omphalocele, visceromegaly, hypoglycemia and elevated risk of embryonal tumors characterize Beckwith-Wiedemann syndrome (BWS), genetically heterogenous disorder caused by dysregulation of imprinted growth regulatory genes within the 11p15 region. The molecular defect underlying a significant proportion of BWS cases remains unknown.
Although Sotos and BWS are clinically distinct conditions, they share common features such as macrosomia, neonatal hypoglycemia or cardiac and genito-urinary anomalies. Based on this phenotypic overlap, we tested whether unexplained Sotos cases could be related to 11p15 anomalies and whether unexplained BWS could be related to NSD1 aberrations. Indeed, 2 anomalies of the 11p15 region were identified in a series of 19 Sotos patients carrying no NSD1 aberration. On the other hand, we identified 2 NSD1 mutations in 51 BWS children with no 11p15 anomaly. These results suggest that the two disorders may have more similarities than previously thought, based on molecular genetic findings. We propose that NSD1 protein could be involved in imprinting of the chromosome 11p15 region.

C06. Mutations in the VKORC1 gene cause warfarin resistance and multiple coagulation factor deficiency type 2

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Coumarin drugs are the therapy of choice for treatment and prevention of thromboembolic events. Coumarins target blood coagulation via inhibition of the vitamin K epoxide reductase complex (VKOR). This complex recycles vitamin K 2,3-epoxide to vitamin K hydroquinone, an essential cofactor for the post-translational gamma-carboxylation of several blood coagulation factors. VKOR has been purified only paritally, and its gene has remained elusive.
We hypothesized that VKOR is involved in two heritable diseases, combined deficiency of vitamin K dependent clotting factors type 2 (VKCFD2) and resistance to coumarins (warfarin resistance WR). WR has been mapped in rats in mice. We localised VKCFD2 by homozygosity mapping to the pericentromeric region of chromosome 16. Using these mapping information from three species, we performed a systematic mutation screen. Mutations in 2 VKCFD2 patients, 4 WR patients and a WR rat strain identified a small transmembrane protein of the endoplasmic reticulum, named vitamin K epoxide reductase complex subunit 1 (VKORC1). Recombinant expression of the wild-type protein, but not the protein carrying the VKCFD2 mutation leads to a striking increase in VKOR activity which is sensitive to warfarin inhibition.
VKORC1 is the first component of the VKOR complex to be identified at the molecular level. Mutations in VKORC1 contribute to understanding the pathomechanism of VKCFD2 and WR. Our findings may also provide a basis for a rational design of novel anticoagulant drugs targeting VKOR.

C07. Identification of exonic splicing regulatory elements in CFTR and BRCA1 genes

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When sequence variants are identified in genomic DNA, especially during routine analysis of disease-associated genes, the full implication of the molecular effect of the substitution might not be immediately evident.Exonic sequence variations can result unexpectedly in disease or mutant phenotype by affecting splicing. The critical issue is to identify if a given nucleotide change is a polymorphism or a splicing mutation. We have analyzed the CFTR and BRCA1 to explore the frequency and mechanism by which exonic substitutions affect splicing. Several changes in CFTR exon 12 (missense and silent) induce a variable extent of exon skipping. This phenomenon is due to the interference with a new regulatory element, the Composite Exonic Regulatory Element of Splicing (CERES). On the other hand, in BRCA1, a G to T substitutions in exon 18 was shown to causes exon skipping and the mechanism still controversial will be discussed. Multiple synonymous substitutions in exon 12 lead to changes in the splicing efficiency suggesting that the codon bias may be the result of natural selection of splicing, The effect of single nucleotide substitutions at CERES and at the BRCA1 exon 18, or changes at synonymous codons, were not predicted by SR matrices.
Exonic variations that create new splicing regulatory elements or that affect CERES may represent a frequent disease-causing aberrant splicing defects. Our results also indicate that even the most benign looking polymorphism in an exon cannot be ignored as it may affect the splicing process.

C08. Is the mandibuloacral dysplasia, a progeroid laminopathy, a new chromatin remodelling disease?

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Chromatin remodelling influence a succession of processes including DNA modifications, covalent histone modifications, and histone relocation. These processes control the chromatin structure and thereby gene expression, DNA methylation, replication, recombination, repair, apoptosis and senescence. Numerous evidences have established the relationship between epigenetic changes and the molecular pathogenesis of several monogenic diseases involving chromatin remodelling. The mandibuloacral dysplasia (MAD, OMIM 248370) is caused by a mutation in LMNA-encoding lamin A/C. The lamins exert a profound influence in the maintaining the structural integrity of the nuclear lamina and on the organization of heterochromatin within the nucleus. In order to study the nucleus architecture, we examined the nucleus morphology, the cytoarchitecture, the chromatin organization and the expression of its associated proteins in fibroblast cell lines from MAD patients carrying the R527H homozygous mutation. Nuclear alterations mostly consisting with focal absence of peripheral heterochromatin were detected in non-degenerating fibroblasts. In the areas devoid of peripheral heterochromatin, the nuclear lamina showed an irregular thickness when compared with normal areas. The major protein component of heterochromatin, heterochromatin protein 1 (HP1), and histone H3 Lys9 methylated (H3Lys9) were found strongly reduced in patient's cells compared to controls. Because HP1 is an integral component of H3 Lys^9-methylated chromatin, we suggest that this reduction is a consequence of an impaired chromodomain function. In fact, no differences at RNA levels were detected for genes encoding these proteins. These results strongly suggest that chromatin remodelling is a key event in the cascade of epigenetic events causing MAD and possibly other laminopathies.

C09. Impaired calmodulin binding of Myosin-7A causes autosomal dominant hearing loss (DFNA11)

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We identified a heterozygous missense mutation (R853C) in a family with autosomal dominant non-syndromic hearing loss that changes an evolutionarily invariant residue of the fifth IQ motif (IQ5), a putative calmodulin (CaM) binding domain, of myosin-7A (MYO7A). Functional effects of the R853C mutation were investigated in a physiological cellular environment by expressing wildtype or mutant MYO7A IQ5-containing peptides in smooth muscle cells of microarteries. Overexpression of wildtype IQ5 (with intact calmodulin binding) would be expected to compete for CaM with myosin light chain kinase (MLCK). Indeed, analysis of calmodulin-dependent vasoconstriction suggests constitutive binding of the wildtype, but not the R853C-mutated, IQ5 motif to CaM at all physiologically relevant Ca2+ concentrations. Both CaM and MYO7A are mandatory for transduction and adaptation processes in inner ear hair cells. A role of myo7a in slow adaptation has been suggested based on investigation of the congenitally deaf mouse mutant shaker-1 resulting from recessive deleterious myo7a mutations. Our data suggest a disturbed CaM/MYO7A binding in R853C heterozygotes. This defect may represent the pathogenetic mechanism resulting in impaired adaptation to environmental stimuli and progressive deterioration of hearing transduction. Our results support the still controversially discussed view that, in addition to its role in providing structural hair cell integrity, MYO7A is a constitutive element of the hair bundle's adaptation machinery. Importantly, the CaM/MYO7A interaction represents an attractive molecular target for therapeutical interventions aimed to delay or prevent the onset of hearing loss in families with mutations in myosin IQ domains.

C10. SCA14 in the Dutch ataxia population

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ADCAs are a clinically and genetically heterogeneous group of neurodegenerative disorders. A molecular analysis in the SCA1, 2, 3, 6 and 7 genes cannot be made in one-third of the ADCA families. One way to identify additional SCA genes is to use linkage analysis. However, this requires large pedigrees and most of the Dutch ADCA families are too small. We have therefore used an alternative approach.
Recently we were able to demonstrate the existence of detectable founder effects for the SCA3 and SCA6 genes in the Netherlands. Therefore, we believed there may well be founder effects for the other SCA loci for which no specific gene or associated mutation have yet been identified. To make use of this phenomenon, we set out to use a shared haplotype analysis (SHA) to localize and identify novel SCA genes in the remaining SCA loci.
We report a six-generation Dutch ADCA family in which linkage to the SCA14 locus on chromosome 19q13.4-qter was found by using SHA. Recently, missense mutations in exon 4 of the PKCgamma gene were reported in 2 ADCA families. We sequenced the PKCgamma gene and identified a novel missense mutation in exon 4. Alternatively, we sequenced the complete Dutch ataxia population and identified additional individuals whom showed the Gly118Asp mutation. Genealogical research was able to link all individuals to the initial large SCA14 family. This finding strengthens our hypothesis that seemingly independently referred ADCA families can be linked to larger pedigrees by combining genealogical research with a shared haplotype approach.

C11. *PA26* is a candidate gene for human heterotaxia

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Heterotaxia is a congenital disorder characterised by a reversed left-right polarity of one or more organ systems and is often associated with congenital heart defects. Whereas several genes involved in left-right axis determination have been identified in animal models little is known about the genes controlling this process in humans. We here report on the identification of a candidate gene for human heterotaxia and on functional studies of this gene in zebrafish.
The gene *PA26* was disrupted by a translocation breakpoint in a patient with heterotaxia and a *de novo* reciprocal translocation t(6;18)(q21;q22). Northern blot analysis showed a decreased expression of the *PA26* gene in an EBV-cell line of this patient. Mutation analysis of the human *PA26* gene in 40 unrelated individuals with unexplained heterotaxia failed to identify mutations. Analysis of the *PA26* gene structure resulted in the identification of a novel *PA26*-related gene family which we named the sestrin family, comprising the three closely related genes in humans as well as in mouse and in zebrafish. We identified the *PA26* zebrafish orthologue and proved syntheny with the human *PA26*. By means of morpholino knockdown technology we showed an alteration in the situs of the pancreas in 35% of the knockdown zebrafish embryos. We conclude that our zebrafish knockdown model supports a role of *PA26* in human situs determination.

C12. Heterozygous missense mutations in *BSCL2* are associated with distal hereditary motor neuropathy and Silver syndrome

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Distal hereditary motor neuropathy (dHMN) or distal spinal muscular atrophy (OMIM #182960) is a heterogeneous group of disorders characterized by an almost exclusive degeneration of motor nerve fibers, predominantly in the distal part of the limbs1. Silver syndrome (OMIM #270685) is a rare form of hereditary spastic paraparesis mapped to chromosome 11q12-q14 (*SPG17*) in which spasticity of the legs is accompanied by amyotrophy of the hands and occasionally also the lower limbs2,3. Silver syndrome and most forms of dHMN are autosomal dominantly inherited with incomplete penetrance and a broad variability in clinical expression. A genome-wide scan in an Austrian family with dHMN-V (ref. 4) showed linkage to the locus *SPG17*, which was confirmed in 16 additional families with a phenotype characteristic of dHMN or Silver syndrome. After refining the critical region to 1 Mb, we sequenced the gene Berardinelli-Seip congenital lipodystrophy (*BSCL2*) and identified two heterozygous missense mutations resulting in the amino acid substitutions N88S and S90L. Null mutations in *BSCL2*, which encodes the protein seipin, were previously shown to be associated with autosomal recessive Berardinelli-Seip congenital lipodystrophy5 (OMIM #269700). We show that seipin is an integral membrane protein of the endoplasmic reticulum (ER). The amino acid substitutions N88S and S90L affect glycosylation of seipin and result in aggregateformation leading to neurodegeneration.

C13. Identification and detailed characterization of intragenic human single-exon genes, that arose late in evolution

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Goal: We want to show that retropositional gene formation has been a valuable source for novel genes in late evolution, i.e. after the whole genome duplications had already taken place and the ray-

finned fish (Actinopterygii) had been formed.
Approach: 1326 single-exon genes are listed at the "UCSC Genome Informatics Site". We screened all these genes for those that reside within an intron of another, well-defined, multi-exon gene (called "host-gene"). In order to find those single-exon genes that arose recently (i.e. do definitely not exist in the puffer-fish Fugu), we identified the Fugu orthologs of the human host-genes (by amino acid similarity and by syntheny of the neighbouring genes) and checked the introns of these host-gene orthologs for the presence of the corresponding single-exon gene.
Results: Up to now, we have identified 117 intragenic human single-exon genes, many of which are definitely absent from the Fugu genome, i.e. have been generated recently. Furthermore, these genes have a clear, spliced precursor gene, and so it is very likely that they arose from a retrotransposition event. Examples for such novel, intragenic single-exon genes are: ***CHML*** (derived from CHM / invaded into OPN3 / absent in the Fugu OPN3 orthologue / essential biological functions in mammals, implicated in Usher-Syndrome type II); ***LDHL*** (derived from LDHA / invaded into Myo1E / absent in the Fugu Myo1E orthologue / expressed in human brain) and ***OXCT2*** (derived from OXCT / invaded into BMP8 / absent in any of the Fugu BMPs / expressed in human brain).

C14. Microarray analysis of fetal human neocortex: candidate genes for brain development and function

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Development of the human neocortex depends on spatially and temporally correct expression of numerous genes. Disturbances of this highly coordinated process are an important cause of mental retardation, epilepsy and many other brain disorders. It is plausible that the same set of genes also contributes significantly to cognitive variation. Identification of genes involved in human brain development and characterization of their spatiotemporally regulated expression patterns may provide a better understanding of cognitive processes. Microarrays allow monitoring the expression of numerous candidate genes for cognition during human brain development in parallel. To this end we have developed a cDNA chip with approximately 600 genes that are known to influence some aspect of cognition in humans, mice and/or Drosophila, along with 100 control house keeping genes. This customized gene chip is used to quantify the mRNA expression levels in fetal (weeks 15-25 of gestation) brain samples from frontal cortex (prospective area A10). Approximately 300 genes on the chip displayed detectable expression levels.
A subset was expressed differentially at different time points of gestation. In addition, we showed distinct expression patterns in brains from Down syndrome fetuses. Microarray results will be validated with reverse Northern blots and real time PCR. Because the topography of gene expression during early human cortical development is largely unknown, immunolocalisation the protein products of genes of interest in fetal brain sections to specific cell types is underway. Collectively, our results will provide new insights into the genetics of human brain development (corticogenesis) and cognitive processes.

C15. Gene Expression Variation In The Human Genome: Dissecting Regulatory Variation.

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Extensive sequence variation is present in human populations, but only a fraction of this is of functional significance. One way in which sequence variation can be functionally active is by modulating gene expression. Correct RNA levels are important for the proper function of a cell and many human disorders are a result of changes in the abundance or pattern of specific transcripts. Recent studies have reported significant gene expression variation in humans, mice and other organisms, and an important fraction of this variation appears to be genetically determined. Natural variation in gene expression is suspected to be involved in the etiology of complex disorders, and is likely to serve as useful intermediate markers, providing a more direct link between sequence variation and phenotypic outcomes.
We are investigating the extent and pattern of gene expression variation in forty-one chromosome 21 genes, using lymphoblastoid cell lines from the CEPH families collection. The genes were selected on the basis their expression in lymphoblastoid cells, and were studied 6 CEPH Utah families. Precise mRNA quantification was performed using Taqman real-time PCR and the Illumina system. We performed 6 replicates for each measurement in order to obtain accurate error calculations.
We will present our results concerning the levels of gene expression variation observed, the heritability of the gene expression differences, genome-wide eQTL mapping, and a comparison between Taqman and Illumina methodologies. These results provide a first step for identifying the molecular mechanisms underlying gene expression variation.

C16. Functional analysis of Conserved Non-Genic sequences (CNGs)

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The description of the functional characteristics of the various sequences of the human genome is of primary importance for the understanding of the contribution of each sequence variant to human disease phenotypes. A powerful way to recognize functionally important sequences is by comparative analysis of various genomes. Our comparison between the entire 33.5 Mb long human chromosome 21 and its mouse homologous regions for sequences with >70% identity over >100bp, revealed that there exist a large number of conserved genomic regions that are not protein-coding genes. More recently, we have shown that these conserved non-genic sequences (CNGs) are significantly more conserved than protein coding genes and noncoding RNAs. The function of these regions is unknown, but they may be involved in transcription, chromosome replication, or structural elements of chromosomes.
In order to assess for the potential functions of the CNGs, we are currently testing a fraction of these CNGs for their ability to modulate transcription of a reporter gene (luciferase assay), their ability to interact with mammalian proteins (1-hybrid assay, ChIP-chip and bandshift experiment) and their DNase I hypersensitivity. Additional experiments include chromatin structure analysis (DNA methylation and histone modification). Preliminary results suggest that transcriptional regulation may not be the main role of the conserved sequences. Interestingly some CNGs may function through binding with proteins, and this binding is in some cases cell type-specific.

C17. Evolutionary fragile sites: implications for hominoid chromosome evolution

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Comparative FISH mapping of some 150 large-insert clones revealed at least 14 independent evolutionary breakpoints between human chromosome 3 and the genomes of five representative primate species. Many rearrangements, i.e. the pericentric inversions leading to orangutan 2, were not simple breakage and reunion events, but involved microduplications and/or microdeletions. The breakpoint regions that distinguish human 3p25.1, 3p12.3 and 3q21.3 from orangutan 2 contain paralogous sequence blocks, which were also duplicated at many subtelomeric regions during hominoid genome evolution. FISH of fully integrated BAC maps to orangutan, siamang gibbon and silvered-leaf monkey chromosomes combined with precise breakpoint localization by PCR analysis of flow-sorted chromosomes localized three independent rearrangements of the human 3q21.3-syntenic region within a <250 kb BAC contig. Approximately 200 kb of the human 3q21.3 segment were not present on the homologous primate chromosomes, suggesting a genomic DNA insertion into the breakpoint region in the lineage leading to

humans and African great apes. The inserted segment represents part of an ancestral duplication of 3q21.3-paralogous sequences. The three breakpoint regions between human 3p25.1, 3p12.3, 3q21.3 and orangutan 2 also represent breaks of chromosomal synteny between the human and rodent genomes. Collectively our data suggest reuse of the same short fragile sites in primate and mammalian evolution. Large-scale chromosome rearrangements, microduplications and microdeletions can be considered as different aspects of an inherent instability of these regions. Evidently, genome architecture, in particular low-copy repeats have played an important role during speciation.

C18. In silico functional analysis of human disease associated genes

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We present an in silico functional analysis of the human genome by combining computer-assisted text mining with automatic and manual annotation of the human genome. Our approach combines results from automatic text mining with manual and automatic functional annotations of human genes with respect to functional categories (including biological process, molecular function and localization) and domains of gene products. Such data integration allows the computation of multilevel correlations among diverse, independent experimental information. These multilevel correlations provide stronger evidence for causal relationships among genes than correlations based on single experimental approaches. In addition, new and unexpected correlations may be identified.
We integrated automatic and manual annotations of approximately 40,000 genes in the Biomax™ Human Genome Database (Schüller and Fritz, 2002) with results generated by the Biomax text-mining tool „Relate“. The text-mining tool preprocesses any text database using a specific knowledge base to standardize and summarize the contents. We have analyzed the complete MEDLINE database for human genes associated with diseases using the text-mining tool. We will discuss the distribution of the disease-associated genes with respect to functional categories and protein domains.
Endy and Brent (2002). Modelling cellular behaviour. *Nature*, **409**, 391-395
Schüller and Fritz (2002). An enhanced Human-Genome Database. *GEN* **22**,38

C19. Developing a screening tool for complex diseases using multiple genetic tests: how many genes are needed?

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Introduction: Population screening tools for complex diseases will need to include multiple genes, since the effects of single genes are weak. We aim to determine how many genes are needed to develop a useful tool.
Methods: Usefulness was evaluated by the accuracy of the tool in discriminating between (future) patients and non-patients, which is indicated by the area under the ROC curve (AUC). AUC ranges from 0.5 (no discrimination) to 1.0 (perfect discrimination). The discriminative ability was investigated for different combinations of disease frequency (d), risk allele frequency (f) and odds ratio (OR) of the risk allele. For each combination, we simulated a population of 100000 subjects using S-Plus software.
Results: When the frequency of disease and risk alleles are both 10%, at least 8 genes with strong effects (OR = 4.0) are needed in the tool to obtain good discriminative ability (AUC = 0.80). At least 33 genes are needed when all ORs are 2.0, and 99 genes when ORs are 1.5. For multiple genetic tests that include genes with different frequencies and ORs, the discriminative ability is higher when ORs and frequencies of risk alleles are higher. Each additional gene has a smaller effect on the discriminative ability of the tool, also in the hypothetical case that all ORs are equal.
Conclusions: Good discriminative ability can be obtained when the screening tool includes genes that are strongly associated with the disease. When all genes have weak effects, a considerable number will be needed to obtain sufficient discriminatory power.

C20. Human sequence variation and disease - The HapMap project

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The HapMap project aims to generate a haplotype map of the human genome, a tool that will facilitate disease and other genetic studies. The project's study-design includes four population samples namely CEPH and Yoruba trios as well as unrelated Japanese and Chan Chinese. The consortium adopted a hierarchical strategy and in the first phase is generating a map of evenly spaced SNPs (1 per 5kb) with minor allele frequency >= 0.05. Assessment of local LD patterns in each population will identify both haplotype blocks and regions that require additional SNPs. The latter will be the focus of phase 2; we anticipate testing over 1.6M SNPs in total. Data are being released regularly at http://www.hapmap.org; the January release figures data on 323,723 SNPs.
Sanger's contribution to the HapMap project includes chromosomes 1, 6, 10, 13, and 20. Genotyping is carried out on the Illumina platform (1536-plex reactions). A study of 10,000 SNPs across 20q12-13.2 was used to assess accuracy vs multiplexing; we estimated that the average error rate is <0.3% and is not influenced by the level of multiplexing.
In parallel, we have undertaken an in depth analysis of chromosome 20. 60,000 SNP assays were designed and genotyped at Illumina across multiple populations. We obtained over 30,000 SNPs with m.a.f >=0.04 and are in the process of assessing common patterns of LD, haplotype blocks and recombination.
We are also conducting a case/control association study for type II diabetes with 4608 of the validated SNPs in 20q12-13.2.

C21. Comparative haplotype diversity among European populations: implications for gene mapping studies

***J. C. Mueller**[1], E. Lohmussaar[1], R. Mägi[2], M. Remm[2], T. Bettecken[1], P. Lichtner[1], T. Illig[3], P. Pramstaller[4], G. Romeo[5], H. Wichmann[3], C. Zoccali[6], A. Metspalu[2], T. Meitinger[1];*

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Linkage disequilibrium (LD) is fundamentally important in association studies, whereby disease variants can be detected through correlation with adjacent markers. The aim of this project is to compare the LD patterns in European populations with respect to gene mapping approaches. We selected several genomic regions adding up to more than one Mb of sequence, which contain candidate genes for complex traits. Evenly distributed markers with an average spacing of about 2-5 kb were genotyped in 8 population samples across Europe including standard control collectives. The CEPH trios of the HapMap project are used as a reference population.
We assessed the heterogeneity in LD block boundaries and haplotype frequencies. We show that the main LD block structure is similar across populations and common haplotypes occur in all populations. In contrast frequencies of single haplotypes can differ considerable among populations. We therefore estimated the proportion of haplotypes we would miss in each population when haplotype tagging markers are selected only in the reference population. We propose recommended tresholds for marker densities and strategies for haplotype tagging in order to support the design of European association studies.

C22. Investigation of Genetic Variation in 111 Candidate Genes for Drug Response Shows Relevance of Rare Variants

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Pharmacogenetic studies searching for genetic factors that may underlie inter-individual differences in drug response require the knowledge of variation in candidate genes. In order to identify single

nucleotide polymorphisms (SNPs) and analyze their characteristics in a set of 111 candidate genes, we re-sequenced exons and flanking regions covering more than 292 kb of genomic regions in an average of 170 chromosomes of European origin. A total of 729 SNPs were identified. SNPs predicted to be functionally important by changes in the amino acid sequence of the encoded protein were enriched among rare variants. Only a small fraction of these rare variants is represented in the public SNP databases illustrating the need of re-sequencing. This might be true especially for the investigation of severe side effects, for which rare variants may be plausible candidates. In non-coding regions, a lower minor allele frequency was observed for SNPs in interspecies conserved regions compared to non-conserved regions, suggesting that SNPs in conserved regions include a significant number of functional variants. For each gene locus we computed htSNPs that capture common haplotype diversity. Although the knowledge of htSNPs reduces the genotyping effort to capture common haplotype diversity, for certain genes this may only account for a low fraction of total haplotype diversity.
In conclusion this study shows that re-sequencing is important for pharmacogenetic hypotheses, particularly with respect to side effects of drug, since individual drug response appears to be preferentially determined by rare variants.
Supported by the German Human Genome Project (DHGP).

C23. A large-scale association study reveals novel breast cancer susceptibility genes

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Over 210,000 individuals are diagnosed with breast cancer annually in the U.S., with an expected death rate of nearly 20%. For more than 10 years, two genes (BRCA1, BRCA2) have been known to harbor highly penetrant mutations that increase breast cancer susceptibility many-fold. However, very few genes have been identified that influence risk of sporadic breast cancer, which represents the majority of incident cases (>90%). In an attempt to identify genes that influence breast cancer risk, we tested over 25,000 single nucleotide polymorphisms located within 10 kb of nearly 50% of known and predicted genes. Using 272 cases and 276 age-matched controls of German descent, we identified over 50 gene regions with evidence of breast cancer association. One of the genes identified is DLC-1, a tumor suppressor gene previously described as a candidate for sporadic breast cancer. Replication of these regions in two independent case-control collections provided additional genetic support for several novel candidates. One of the regions contained a cluster of genes encoding adhesion molecules that has been previously suggested as being involved in the etiology of several forms of cancer. In an independent study we demonstrated that the breast cancer-associated alleles are also associated with prostate cancer. We are currently characterizing the patterns of haplotype variability and re-sequencing targeted regions to identify the genetic variations that are etiologically responsible for disease risk. Furthermore, we demonstrate how these combinations of variations, each with modest, marginal influences on disease risk, can be useful for predicting individual risk in clinical practice.

C24. High Resolution LD-mapping of the Myocardial Ikr Channel Subunit Gene KCNQ1 reveals two independent Effects of Gene Variants on the QT-Interval

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AIM: Cardiac arrythmias are frequently caused by delayed repolarization under control of voltage gated potassium channels. Extreme phenotypes are the monogenic long QT Syndromes (LQT1-LQT6). We undertook LD mapping of the KCNQ1 Gene (LQT1, 400kb, alpha-subunit of myocardial Iks current) followed by association analysis of frequent gene variants with QT-interval in the general population. METHODS: In n=702 population based probands we recorded ECGs and calculated a QT-time corrected for age, sex and heart rate (QTCN). Probands were genotyped for 61 SNPs in a 600 kb region. LD-structure was characterized by determining LD measures D‘ and r2. Haplotypes in the individual blocks of LD were determined statistically. Probands with ventricular pacemakers, complete bundle branch blocks or brady- or tachyarrhythmia were excluded from association analysis. In the remaining probands (n=657) the corrected QTCN-interval was associated with genetic variants. RESULTS: The genomic region of the KCNQ1 gene extends over 10 haplotype blocks measured at a coverage of 1 SNP / 10kb. The strongest association to the QTCN-interval was between two SNP markers in Intron 1 (AFmin = 0.37) and in Exon 15 (AFmin = 0.10) (p=0,0085 and p=0,02 resp.) explaining 1,52% and 0,87% of the entire variance of QTCN. Both SNPs were in complete linkage equilibrium and independent with respect to their effects (p=0.004). Their combined effect explained 2,25 % of the variance of QTCN. CONCLUSION: In the genomic region of the KCNQ1 gene at least two frequent genetic variants exist in separate LD-blocks that independently and additively influence myocardial repolarization.

C25. Shprintzen-Goldberg Syndrome: Fourteen New Patients and a Clinical Analysis

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The Shprintzen-Goldberg syndrome (SGS) is a disorder of unknown cause characterized by craniosynostosis and a marfanoid habitus, as well as skeletal, neurological, cardiovascular, and connective-tissue anomalies. There are no pathognomonic signs for SGS and diagnosis depends on recognition of a characteristic combination of signs.
In this report we describe 14 persons with SGS and compare their clinical findings with those of 23 previously reported individuals. Analysis of the 14 persons with SGS presented in this work together with the 23 cases previously described in the literature lets a relatively comprehensive view of the clinical and radiographic abnormalities commonly or occasionally seen in persons with SGS emerge, which may be useful for clinical decision making. There is a characteristic facial appearance in SGS, with more than two thirds of all individuals having hypertelorism, down-slanting palpebral fissures, a high-arched palate, micrognathia and apparently low-set, posteriorly rotated ears. Other commonly reported manifestations include hypotonia in at least the neonatal period, developmental delay, and inguinal or umbilical hernia. The degree of reported intellectual impairment ranges from mild to severe. The most common skeletal manifestations of SGS were arachnodactyly, pectus deformity, camptodactyly, scoliosis, and joint hypermobility. None of the skeletal signs alone is specific for SGS.
Our study includes 14 mainly German individuals with SGS evaluated over a period of 10 years. Given that only 23 other persons with SGS have been reported to date worldwide, we suggest that SGS may be more common than has been previously assumed.

C26. Autosomal recessive congenital cutis laxa: further evidence for heterogeneity and delineation of the Debré type with eight new cases

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Autosomal recessive cutis laxa has been subdivided until now between a type with pulmonary emphysema and a type with growth retardation and dislocation of the hips. We describe eight new patients of the latter type and provide a critical review of the 45 similar clinical reports published until now under the names of cutis laxa or wrinkly skin syndrome. This lead us to reconsider the nosology of congenital cutis laxa and to suggest splitting of the growth retardation subtype in Debré type with megafontanelles and a distinctive facial dysmorphia (that includes telecanthus and downward slant of palpebral fissures) and another type lacking these features. Differences between the pulmonary emphysema and the Debré subtypes in terms of life expectancy, intellectual development and cutaneous phenotype indicate a poor prognosis, normal intelligence and worsening of the cutis laxa in the former while prolonged survival, moderate to severe mental retardation and improvement of cutis laxa are the rule in the latter. Central nervous system involvement is also exemplified by the occurrence of brain dysgenesis (frontal polymicrogyria) and intractable seizures in a female patient and Dandy-Walker malformation in another one. Interestingly, an unreported 'Ehlers-Danlos like'phenotype with pseudo-ecchymotic pretibial skin lesions was observed in two unrelated affected patients.

C27. Multicentric study of Baraitser-Winter syndrome: critical review, further delineation and re-definition in 19 patients

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Baraitser-Winter syndrome is an exceptional disorder characterized by short stature, hypertelorism, broad epicanthus, bilateral ptosis, coloboma, metopic ridge and pachygyria. Fryns-Aftimos syndrome is defined by hypertelorism, ptosis, large nose, neck webbing, low posterior hairline, broad thorax, frontal pachygyria, preaxial anomalies and seizures. An unusual body habitus is present, with truncal obesity, tip-toe walking, limited extension of knees and shoulder propulsion. Both disorders are reminiscent of Noonan syndrome and both are usually associated with mild to severe mental retardation.

We report a series of 19 patients (including update on 4 previously reported cases) with a phenotype overlapping Baraitser-Winter and Fryns-Aftimos syndromes, suggesting that both disorders are identical (but reported at different ages: youngsters in Baraitser-Winter, teen-agers in Fryns-Aftimos) and possibly less exceptional than previously thought. PTPN11 screen was negative in 3 of our cases, and telomere screen in several of them. Facial Gestalt of Baraitser-Winter seems the most reliable clue for diagnosis. Predominantly frontal pachygyria and coloboma are common but inconstant. Urinary tract defects, bifid hallux, deafness are repeatedly reported. Mental retardation is variable and may be influenced by epilepsy and/or cortical defect. Contrasting with Noonan syndrome, heart is not a target. Most cases are sporadic. Based on this new series, we review the earlier literature and propose a renewed definition of Baraitser-Winter syndrome, discussion boundaries with C syndrome, CHARGE association, LE-Marec-Odent-Urvoy or Mégarbané syndromes, and the frontofacionasal syndromes.

C28. Homozygous missense mutation in the lamin A/C gene causes autosomal recessive Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder with children displaying features reminiscent of premature senescence. Recently, heterozygous, recurrent *de novo* point mutations in the *LMNA* gene encoding lamin A/C, a component of the filamentous meshwork of the nuclear lamina, have been reported to cause HGPS, supporting the prevailing hypothesis that HGPS represents a sporadic autosomal dominant disorder. In this study on a consanguineous HGPS family we provide molecular evidence for autosomal recessive inheritance of HGPS. Genome-wide linkage analysis, performed before the recent identification of the gene excluded all chromosomal regions except for 1p13.3-1q23.3 where the *LMNA* gene is located. Subsequent screening of the HGPS family indicated that all 4 living affected children share the same homozygous missense mutation G1626C (K542N) in *LMNA*. Both parents as well as one healthy daughter were found to be asymptomatic, heterozygous K542N mutation carriers. Besides HGPS, germline mutations in *LMNA* have been shown to cause 7 different dominantly and/or recessively inherited disorders. Our findings indicate that HGPS represents, in addition to the Emery-Dreifuss muscular dystrophy (EDMD), the second laminopathy in which germline mutations in the Lamin A/C gene can cause disease in both a dominant and recessive mode of inheritance. Given the phenotypic overlap commonly observed among the laminopathies and the extent of skeletal lesions present in this HGPS kindred, our observations raise the question whether autosomal recessive mandibuloacral dysplasia (MAD) and HGPS represent essentially the same genetic disorder albeit with varying degrees of disease severity.

C29. Clinical and Mutational spectrum of Mowat-Wilson syndrome

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Mowat-Wilson syndrome (MWS) is a distinct recognizable multiple congenital anomalies-mental retardation syndrome caused by mutations or deletions in the zinc finger homeo box 1B gene. Until now 53 patients have been published with deletions or truncating mutations of *ZFHX1B*. Common phenotypical aspects are distinct facial dysmorphism and severe mental retardation in all cases, seizures in 77%, Hirschsprung disease in 72%, congenital heart defects in 48%, agenesis of corpus callosum in 49% and variable multiple congenital anomalies.

However, Yoneda et al. (2002) described a patient with milder and late-onset mental retardation and absence of Mowat-Wilson facial gestalt, who had a novel in frame 3-bp deletion.

In order to further delineate the clinical and mutational spectrum of Mowat-Wilson syndrome we analysed 31 patients with variable symptoms of the MWS spectrum. While 14 had the characteristic facial phenotype and other typical multiple anomalies, the remaining patients had either questionable facial features (4) or non-specific dysmorphism and clinical features (13).

In the group of "typical" Mowat-Wilson syndrome patients we detected *ZFHX1B* deletions or nonsense mutations in 13 of 14 patients. 11 of the mutations are new and distributed over the whole coding region. In the cases without the typical Mowat-Wilson facial

phenotype and with questionable or non-specific clinical symptoms we could identify neither deletions nor missense- or nonsense mutations. In 9 patients we found three common and three rare SNP´s.
Thus we further confirm that at least null-or nonsense mutations of the *ZFHX1B* gene result in the distinct, clinically well recognizable Mowat-Wilson syndrome.

C30. Clinical variability of Cohen and Mirhosseini-Holmes-Walton syndromes caused by mutations in COH1

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Cohen syndrome is a rare autosomal recessive disorder whose diagnosis is based on a variable clinical picture of psychomotor retardation, microcephaly, typical facial dysmorphism, progressive pigmentary retinopathy, severe myopia, and intermittent neutropenia. Recently, mutations of the gene COH1 were identified in a Finnish and Northern European cohort of patients with Cohen syndrome. The present study describes the phenotypes in 20 patients with Cohen syndrome from 12 non-Finnish families, originating from Brazil, Germany, Lebanon, Oman, Poland, and Turkey. These families also included two with an overlapping phenotype of Cohen and Mirhosseini-Holmes-Walton syndromes. All patients were homozygous or compound heterozygous for novel mutations in COH1. The clinical characterisation of the patients analyzed here revealed high variability with developmental delay of variable degree, early onset myopia, and facial dysmorphism as the only features present in all patients, whereas microcephaly, retinopathy at school age, short stature, truncal obesity and neutropenia were lacking in part of them. The diagnosis of Cohen syndrome, particularly based on the typical facial features, was also confirmed in two 24-month old patients, otherwise too young for presenting the complete ophthalmologic phenotype. Identification of COH1 mutations in two families with an overlapping phenotype of both Cohen and Mirhosseini-Holmes-Walton syndromes demonstrated that the two conditions can be in fact allelic. Our findings indicate that mutations in COH1 are also responsible for the Cohen syndrome phenotype in patients from outside Northern Europe and that the phenotype is much more variable than initially proposed.

C31. High prevalence of SLC6A8 deficiency, a novel X-linked mental retardation syndrome

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Recently, a novel X-linked mental retardation (XLMR) syndrome, SLC6A8 deficiency, due to creatine deficiency in the brain caused by mutations in the creatine transporter gene (SLC6A8) (MIM 300036) was identified. The clinical presentation of affected males is XLMR, expressive speech and language delay, epilepsy, and autistic behavior. In approximately 50% of the female carriers, learning disabilities of varying degrees have been noted. Affected males have a reduction of the creatine signal in the proton magnetic resonance spectroscopy (H-MRS) of brain, increased creatine/creaitinine excretion in urine and have impaired creatine uptake in cultured fibroblasts.
We have studied the prevalence of SLC6A8 mutations in a panel of 290 nonsyndromic X-linked mental retardation patients archived by the European XLMR consortium. The full-length ORF and splice sites of the SLC6A8 gene were investigated by DNA sequence analysis. Four missense mutations, 1 single amino acid deletion and one nonsense mutation were identified in a total of 288 XLMR patients, showing a prevalence of at least 2.1% (6/288). Our data indicate that the frequency of SLC6A8 mutations in the XLMR population is close to that of CGG expansions in FMR1 responsible for the fragile X syndrome.
SLC6A8 sequence analysis, creatine/creatinine measurement in urine, magnetic H-MRS of brain, and creatine uptake assay in fibroblasts appear all to be valuable primary diagnostic tests for the evaluation of SLC6A8 deficiency, and should be considered for all males with mental retardation of unknown cause.

C32. Severe X-linked mental retardation caused by mutations in the gene for the thyroid hormone transporter MCT8

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X-linked mental retardation (XLMR) is a highly heterogeneous condition, including >140 syndromic and non-syndromic forms, and there is evidence for pericentromeric clustering of non-syndromic XLMR genes. The gene for the recently identified thyroid hormone transporter MCT8 maps to Xq13 and is a plausible candidate gene for XLMR because thyroid hormones play an important role in the development and function of the brain, and very recently, *MCT8* mutations have been found in patients with complex developmental disorders and mental retardation (Friesema, Visser, Grüters; submitted). Here we report on *MCT8* mutation screening in a large cohort of patients with XLMR from the EURO-MRX Consortium, including patients from 16 families with linkage intervals overlapping the *MCT8* locus and 180 unrelated XLMR patients for whom no linkage information was available. So far, we have found 3 sequence variants in the coding region of *MCT8* which were not observed in normal controls, a 1 bp insertion in exon 1 leading to a premature stop codon in patients with severe MR, hypotonia and seizures, as well as two different missense mutations, which co-segregate with MR in the respective families. To confirm the functional relevance of these mutations, relative concentrations of bound and free serum tri-iodiothyronine will be determined in all three families, and mutation screening is being expanded to include all 450 EURO-MRX families. Depending on the outcome of these studies, screening of serum tri-iodothyronine levels may have to be considered as a fast and non-expensive routine test in all families with XLMR.

C33. Impairment of the renin receptor prevents ERK1/2 activation in a patient suffering from mental retardation and epilepsy

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Within the scope of the DHGP2 project, we have identified and functionally characterized a novel disease gene located in Xp11.4 causing mental retardation-epilepsy syndrome (XMRE). In addition to severe mental impairment, affected members of the XMRE family developed seizures between 4 and 14 months of age. In the course of identifying a candidate gene for XMRE, a gene catalogue for the linkage interval between markers DXS1049 and DXS8054 in Xp11.4 was completed. Large scale mutation analysis revealed a translationally silent variant in exon 4 in a putative exonic splicing enhancer (ESE) site of the ATP6AP2 gene. This variant could not be found in 1200 X chromosomes and results, as demonstrated in

real time PCR experiments, in inefficient inclusion of exon 4 in 50% of ATP6AP2 mRNA in the patient. The ATP6AP2 gene encodes the renin receptor, which binds renin to enhance the cleavage of angiotensinogen. Renin has been known for a long time to be the rate-limiting enzyme for angiotensin peptides generation and the renin-angiotensin system, essential for blood pressure and water-electrolyte control. Functional studies revealed that the mutated receptor failed to activate the MAP kinases ERK1/2, despite binding renin and increasing renin catalytic activity. These findings suggest a specific role for the renin receptor via the ERK1/2 system in brain development and cognitive function. Further studies will focus on the isolation of proteins interacting with the renin receptor to activate in concert with other ligand/receptor systems the MAP kinases ERK1/2. Additionally, knockout experiments in rat are in progress.

C34. New insights from a mouse model for Anderman syndrome

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Anderman syndrome (ACCPN) is an autosomal recessive disorder characterized by agenesis of the corpus callosum, mental retardation, and peripheral neuropathy. It is caused by mutations in the *SlC12A6* gene encoding a K-Cl cotransporter (KCC3) (Howard et al., Nature Genetics, 2002, 32: 384-92). We disrupted *SlC12A6* in the mouse. As in the human disease, *Kcc3*$^{-/-}$ mice show severe motor abnormalities that correlate with a progressive neuronal degeneration in the peripheral and central nervous system. *Kcc3*$^{-/-}$ mice also present with slowly progressive deafness and arterial hypertension, features not described in Anderman syndrome patients so far. Hearing loss is much slower than in mice deficient for the homologous K-Cl cotransporter KCC4, which is specifically expressed in supporting cells of outer hair cells and is essential for ensuring proper ionic environment of outer hair cells. KCC3 was detectable in type I and type III fibrocytes cells of the inner ear K^+ recycling pathway that underlie the stria vascularis. These cells slowly degenerated, as did sensory hair cells. We conclude that KCC3 creates a K^+ gradient within the fibrocyte gap junction system. KCC3 expression in the smooth muscle cell layer of arterial vessels prompted us to measure arterial blood pressure. Indeed, arterial blood pressure was elevated (118±2 mmHg) in *Kcc3*$^{-/-}$ mice compared to control animals (100±2 mmHg; $p < 0.0001$).

C35. A novel open reading frame for the MECP2 gene is mutated in Rett syndrome and defines the MeCP2 isoform relevant to the disease

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Rett syndrome (RTT) is a pervasive developmental disorder characterized by onset, in girls, of a gradual slowing of neurodevelopment in the second half of the first year of life towards stagnation by age four, followed by regression and loss of acquired fine motor and communication skills, and ultimately results in profound mental retardation. Eighty percent of patients with RTT have mutations in exons 3 or 4 of the gene encoding the transcriptional repressor MECP2. In the known transcript of the gene all four exons are utilized, with the translation start site in exon 2. However, we have recently discovered that another isoform of MECP2 exists, namely MECP2B, in which exon 2 is spliced out and translation is initiated from a START codon in exon 1, thus resulting in a slightly larger protein with a very different N-terminal end (Mnatzakanian et al, *Nature Genetics*, April 04). This newly identified isoform of MECP2B appears to be the predominant isoform in brain tissues, with ~10-fold higher expression. In further support of our theory that MECP2B is the etiologically relevant isoform in RTT, we screened 19 females diagnosed with typical RTT, but with no known mutation identified, and identified one patient with an 11 bp deletion within the newly identified coding region, which results in a frame-shift and premature truncation of the protein. Since MECP2A is unaffected by the 11 bp deletion, this confirms that MECP2B is likely to be the etiologically relevant isoform.

C36. Arhgef6-deficient mice, a model for non-specific X-linked mental retardation, show a decrease of mature dendritic spines

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Mutations in *ARHGEF6*, the gene encoding a guanine nucleotide exchange factor for the Rho GTPases Rac1 and Cdc42, are associated with nonspecific X-linked mental retardation. We generated an *Arhgef6* knock-out mouse that will help to elucidate and understand the pathophysiological mechanisms underlying the human phenotype. By Western blot analysis, we showed that the Arhgef6 protein (~87 kD) was present in thymus, spleen, heart, liver, kidney, and brain in wild-type mice whereas the protein was absent in these tissues of the *Arhgef6*-deficient mice. Remarkably, we have also detected four smaller Arhgef6 isoforms in thymus, spleen, liver, kidney, and brain of wild-type mice with molecular weights between 61 and 80 kD. In the *Arhgef6*-deficient mice, we have also investigated spine morphology and density. Spines were visualized by Golgi staining, followed by quantitation of their density along the dendrites of pyramidal neurons in the visual cortex. In *Arhgef6* knock-out mice, the density of mushroom spines (the mature type of spines) along basal dendrites was decreased by 25 %, compared to that of wild-type mice, whereas the density of filiform spines (an immature spine morphology) was not altered. These data are in line with observations made in patients with mental retardation that showed decreased dendritic complexity and dendritic spine density suggesting that mental retardation results from abnormal neuronal connectivity causing deficient information processing. Currently, *Arhgef6*-deficient mice are being tested for behavioural and cognitive deficits.

C37. Detection of subtelomeric rearrangements in 173 patients with unexplained mental retardation using MLPA

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Background: Subtelomeric rearrangements significantly contribute to idiopathic mental retardation and result in several mental retardation syndromes. However the majority of subtelomeric defects lack a characteristic phenotype. In this study we tested the diagnostic capacity of multiplex ligation-dependent probe amplification (MLPA) for screening for subtelomeric aberrations in mentally retarded patients.
Participants and methods: 173 mentally retarded patients (IQ <70), with a normal G banded karyotype at a 550 band level and no clinical recognisable syndrome were tested by MLPA, using a new set of subtelomeric probes, the SALSA P036 Human Telomere Test Kit. Rearrangements were validated and checked for de novo occurrence by FISH. The sensitivity of the technology was characterized on cytogenetically verified positive controls.
Results: Subtelomeric aberrations were detected in 6.4% (11/173) of the patients: 6 deletions (1p [2x], 4p, 15q, 22q [2x]) and 5 duplications (5q, 10q [2x], 12p, 22q). Four deletions and one duplication were validated by FISH, whereas two duplications were not detectable. We are currently in the process of confirming the remaining aberrations and testing the parents of the affected individuals.
Conclusions: This study shows that MLPA analysis is a fast, reliable and relatively inexpensive technique to detect subtelomeric rearrangements. In our series, a subtelomeric aberration was detected in 6.4% (11/173) of the patients. The simplicity of the technique makes it a highly suitable for routine diagnostic screening for submicroscopic telomeric copy number changes in mentally retarded patients.

C38. Application of the Universal Linkage System to genomic microarrays

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Array-based comparative genomic hybridization (arrayCGH) onto BAC arrays (genomic microarrays) is rapidly becoming the method of choice for genome wide DNA copy number screening. We have recently shown that this technology can be applied not only in research settings but also in diagnostic settings because of its high resolution (Vissers et al. 2003). Key issues that determine the success of this approach are the sensitivity and specificity. In addition, the approach should be very robust, high-throughput and cost-effective. The labelling procedure is one of the steps in the arrayCGH procedure that is time-consuming and expensive. In this project we have compared standard enzymatic random-prime labelling with chemical labelling using the Universal Linkage System (ULS^{tm}). We will show that ULS can easily be incorporated in arrayCGH experiments and that it offers several advantages over conventional labelling procedures.

Vissers et al. Array-based comparative genomic hybridization for the genome wide detection of submicroscopic chromosome abnormalities. American Journal of Human Genetics, 73: 1261-1270 (2003).

C39. Microdeletion/duplication scanning using whole genome SNP Chip-Arrays

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Subtle segmental chromosomal aneusomies (SCA) are a major cause of MR/MCA syndromes. With current techniques only a fraction of these aberrations can be detected, mainly those associated with characteristic clinical phenotypes or with subtelomeric rearrangements. New approaches to achieve a higher genome-wide detection rate of such aneusomies are mainly focused on matrix comparative genome hybridisation (CGH). However this technique is still under evaluation and is not routinely available for a high density genomewide screening. Therefore we evaluated an alternative method using commercially available, easy to handle SNP genotyping chip-arrays for parallel interrogation of 11,256 SNPs for the detection of such SCAs. We investigated 20 patients with 21 previously confirmed cryptic aberrations of varying size and location together with both parents. Deletion sizes varied from 192 kb to 12 Mb, duplication size was 5 Mb. While the analysis of mendelian errors in the trios produced a high rate of false positive and false negative results, we were able to detect 11 of 12 deletions and the duplication covered with at least 3 SNPs with a single experiment using the meta-p-value analysis of hybridisation intensities of individual patients' chip hybridisations. One deletion covered by 4 SNPs was only discovered by repeating of chip-array hybridisation. Thus, the 11.5 k SNP Array is already a valuable tool to detect cryptic chromosomal deletions. However, deletions larger than 200 kb should have been detectable using a denser array with 120K SNPs, which is currently under development.

C40. Using a highly-multiplexed approach to quickly score tens of thousands of SNPs and scale up to whole genome studies

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Discovering the genetic basis of complex phenotypes requires new technologies that are sensitive to the full spectrum of human genetic variation and scalable to enable whole genome studies. ParAllele BioScience has developed a suite of highly multiplexed assays to allow researchers to quickly and easily score tens of thousands of SNPs utilizing universal DNA tag arrays for readout, and to scan thousands of target regions for novel mutations in a single-reaction. This platform scales easily to whole genome studies and allows 10-100 times more genotypes to be performed in a single reaction with the flexibility to address any user selected set of SNPs. We will discuss use of the technology and briefly, provide background on the company and founders, previously scientists at the Stanford Genome Technology Center. We will also present results from a case study run in collaboration with a large pharmaceutical company that underlines the importance of discovering and scoring both rare and common variation to identify the genetics associated with complex metabolic diseases. Additionally, we expect to be able to discuss results on the discovery of a gene shown to be associated with Autism. This case control study scored all rare variation as well as mutations in the cohort under a linkage peak.

C41. Gene mapping by linkage analysis with large SNP marker panels

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In the last decade gene mapping was mainly based on genome scans with microsatellite markers. These markers are highly polymorphic but their analysis is technically demanding. Single nucleotide polymorphisms (SNPs) may be a better alternative since their analysis can be easily automated. However, 3-5 SNPs have to be analysed to get equivalent information for one microsatellite. This inflation of markers may cause a risk of stumbling when using current versions of linkage programs. We tested the limitations of standard linkage programs with respect to their capability of analyzing large families with a large number of markers given the limited memory size of common computers. Our test sample comprised genotype data obtained with the GeneChip® Mapping 10K Array (Affymetrix) from several large families segregating either recessive or dominant traits. We used a moving window approach to analyse the genotype data of >10,000 SNPs. A Perl/Tk script (ALOHOMORA) was written which uses the linkage programs Genehunter, GRR, Merlin, Mega2, PedCheck and Simwalk2 for quality control and linkage analysis. Optional filters remove genotypes with Mendelian errors, non Mendelian errors and non informative SNPs. Three genetic maps, DeCode, Marshfield and SLM1 as well as allele frequencies of three ethnic populations, AfroAmericans, Asians and Caucasians can be selected. Using this convenient tool, we have performed >20 mapping projects. We could demonstrate that the information content of the 10K SNP scans was substantially increased as compared with a conventional 400-microsatellite scan. We will present data of several successful genome scans in families with monogenetic diseases.

C42. Parallel Genotyping of over 100,000 SNPs Using a One Primer Assay on a Pair of High Density Oligonucleotide Arrays

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The analysis of single nucleotide polymorphisms (SNPs) is increasingly utilized to investigate genetic causes of complex human diseases. Here we present a high throughput genotyping platform that uses a one primer assay to genotype over 100,000 SNPs per individual on a pair of oligonucleotide arrays. This approach uses two restriction enzymes to fractionate the genome and subsequent amplification of a specific subset of the genome. The resulting reduction in genome complexity enables allele specific hybridization to the arrays. The selection of SNPs was based on computer predictions of restriction fragments that are likely to contain the SNPs, and further driven by strict empirical measurements of accuracy, reproducibility and average call rate, which we estimate to be > 99.5%, > 99.9%, and > 95%, respectively. With median heterozygosity of 0.30 and median inter-SNP distance of 7.98kb across genome, the SNP array provides a viable tool for studying the genetic basis of disease and drug response.

C43. Expression profiling of Wilms tumors reveals novel prognostic markers

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Wilms tumor (nephroblastoma) is the most frequent renal neoplasm in children. It arises from embryonic kidney cells and most frequently presents as unilateral and sporadic tumor. Tumorigenesis is associated with mutations in WT1 and ß-catenin in only a minority of the affected children. Both mutations are frequently associated with each other, but do not have a predictive value.
We performed cDNA microarray experiments using 77 primary Wilms tumors with the aim of detecting new candidate genes responsible for malignancy, tumor progression and prediction of outcome. For a subset of differentially expressed genes microarray data were confirmed by real time RT-PCR on the original set of tumors. The stratification of expression data according to clinical criteria allowed a clear distinction between different subsets of Wilms tumors. Most significant differences in expression patterns were discovered between relapse-free as opposed to relapsed tumors and intermediate risk as opposed to high risk tumors. Several differentially expressed genes, e.g. PRAME, CRABP2, EGR1, CTGF, RARRES3 and EZH2, were identified as important predictors of outcome. CRABP2 and RARRES3 are retinol-related genes and may represent potential therapeutic targets. Our data suggest that expression profiling constitutes a novel molecular tool for prediction of relapse and outcome in Wilms tumors.

C44. Expression analysis of Wilms' Tumor specimens by high density oligonucleotide arrays

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Wilms tumor is the most common cancer of the urinary tract affecting children. In order to determine the gene expression profile Wilms tumor, we analyzed 15 specimens collected in The Children's Hospital at Westmead, Australia between 1989-2001 by high density oligonucleotide microarrays. RNA was isolated from pure tumor cells obtained after laser capture microdissection (LCM). Then the isolated RNA was amplified and labeled using T7 polymerase based in vitro trancription. The hybridization was performed on Affymetrix HU133A GeneChips on which oligonucletides representing ~22500 human mRNA sequences are present. Compared to the reference sample (normal kidney), the expression of 16 genes were found to show two fold or more difference (up-regulated: 9, down-regulated: 7) consistently across all the tumors. The up-regulated genes in tumors are *CRYZ*, *DPYD*, *BLNK*, *ENPP2*, *SEMA3D*, *PTPRB*, *SNAP29*, *KLHL2*, *SAMSN1*, and GJA4. The down-regulated genes are *LTF*, *HES1*, *PDYN*, *GPR51*, *NCAM1*, *TRIM33*, and *AQP3*.

C45. Germline mutations of the paired-like homeobox 2B (*PHOX2B*) gene in neuroblastoma.

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Neuroblastoma (NB, MIM 256700) is a tumour of the sympathetic nervous system that accounts for about 10% of all neoplasms in childhood. Although no predisposing gene(s) have been identified thus far, several lines of evidence support the involvement of genetic factors in NB, namely rare familial cases with vertical transmission and multifocality, and, the association of NB with other genetically determined congenital malformations of neural crest origin such as Hirschsprung disease (HSCR, MIM 142623) and/or congenital central hypoventilation syndrome (CCHS, Ondine's curse, MIM 209880). In particular, CCHS patients have a high predisposing risk to develop a tumour of the sympathetic nervous system (NB, ganglioneuroblastoma and ganglioneuroma, 5-10% versus 1/10,000 in the general population). We recently identified the paired-like homeobox 2B (*PHOX2B,* MIM 603851) gene as the major disease causing gene in CCHS with an autosomal dominant mode of inheritance and *de novo* mutation at the first generation. We therefore regarded *PHOX2B* as a candidate gene in both familial and syndromic NB. Here we report on heterozygous missense mutations located in the homeodomain of PHOX2B in both a familial case of NB and an isolated case of NB associated with HSCR. Although recent results suggest that hereditary neuroblastoma is heterogeneous with the mapping of predisposing genes in familial NB to either 16p or 4p, PHOX2B is the first gene predisposing to NB for which germline mutations have been identified. Further studies are needed to investigate its putative role in sporadic NB.

C46. Expression of *SPOC1*, a novel gene encoding a putative transcriptional regulator, is associated with ovarian carcinoma patient survival

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Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy among women in developed countries, yet little is known about the molecular events that drive the initiation and progression of this disease. Especially for early diagnosis of EOC, prognostic factors, which may be used to identify groups of patients in whom more specific biological treatments or more aggressive therapy is indicated, are of great medical importance.
We report the identification of a novel human gene (*SPOC1*) which encodes a protein with a putative chromatin interacting domain. The gene is located in chromosome region 1p36, a region implicated in tumor development and progression. *SPOC1* mRNA expression was quantified in tumor tissue of 103 patients with epithelial ovarian cancer. Large interindividual differences in *SPOC1* expression were observed. Interestingly, *SPOC1* expression was clearly associated with worse prognosis. The univariable (p=0.001) and multivariable (adjusted to FIGO-stage, residual tumor, histological grade and type: p=0.064) proportional hazards model showed an association between *SPOC1* expression and survival. Median survival time was 1596 days for patients with low *SPOC1* expression versus only 347 days for patients with high expression. Subcellular localization studies of SPOC1 showed a nuclear speckled-like fluorescence that partially co-localizes with RNA-polymerase II. *SPOC1* is strongly expressed in spermatogonia, a rapidly proliferating cell type, and functions as a transcriptional regulator as evidenced by a GAL4-luciferase reporter assay. Thus, the data indicate that *SPOC1* may represent a novel prognostic factor for epithelial ovarian cancer which probably functions as a transcriptional regulator in proliferating cells.

C47. Molecular Genetic Profiling of Normal Prostate, Benign Prostatic Hyperplasia, Dysplasia and Gleason Grade 3, 4 Prostate Cancer

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Prostate cancer (PC) is by far the most prevalent of all human malignancies with the exception of skin cancer. A serum prostate-specific antigen (PSA) level of between 2-10 ng/ml has been widely used in the U.S. as a marker for PC. However, in this range, serum PSA is largely related to benign prostatic hyperplasia (BPH), and correlates poorly with PC curative outcome. Gene expression characterization of prostate cancers could aid in the development of new PC serum markers in the form of an array-based diagnostic. In addition, gene expression analysis of responsiveness to drug treatment for prostate cancer could result in more effective treatment decisions.

More than 80 labeled targets from the prostate central zone, prostate peripheral zone, BPH, Dysplasia, Gleason grade 3 (GG3)and GG4 prostate cancer were hybridized to high-density DNA microarrays, containing probes representing ~22,000 full-length human genes. A group of genes were identified for classification and prediction of different zones of prostate tissue and prostate cancer. The candidates included genes previously associated with PC, as well as genes associated with oncogenesis, transcription, signal transduction and apoptosis. We will explore results obtained from various analysis methods and their relationship to other work. These expression profiles have the potential to improve the staging and sensitivity for detection of prostate cancer within an aging population.

C48. Organizing genetic counselling for cancer in Finland - 10 years experience of the Finnish Cancer Society's three-stage model

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The Cancer Society of Finland, a third sector organization, started in 1994 to organize genetic counselling for cancer in close association with the existing public health care system in the country. The motivation was to offer easy access to counselling at one of the 12 regional Cancer Society Offices to anyone who was worried about their hereditary predisposition to cancer.

At the first level of this three-stage counselling system, a specially trained nurse at one of the regional cancer societies asks for information about the possible cancer cases in the family and draws a pedigree. Using the predefined criteria, the nurse then decides whether the situation requires consultation with a doctor. The notified risk is divided into low, moderate and high. Those with moderate or high risk are referred to the second level where a designated medical doctor asks for more detailed cancer history of the family, and in a high-risk situation sends the patients to a medical genetics unit. At this third level, the actual genetic counselling and possible gene test are organized. All three levels also organize individual follow-ups for each patient according to the risk level.

A great majority of the clients are cared for by the cancer organization nurse at the first level and they do not need any further consultation. Only about 1/3 of the clients are sent to the medical genetics units at university hospitals. In a large and sparsely populated country like Finland a three-stage model like this is democratic and justifiable.

C49. Novel point mutations R173C and A170P in the SHOX homeodomain defines impaired nuclear translocation as a molecular cause for Léri-Weill dyschondrosteosis and Langer dysplasia

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The short stature homeobox gene *SHOX* resides within the pseudoautosomal region of the sex chromosomes and encodes two isoforms of a paired-related homeodomain protein. *SHOX* haploinsufficiency leads to phenotypically heterogeneous short stature conditions including idiopathic short stature and Léri-Weill dyschondrosteosis and is involved in growth retardation and skeletal abnormalities in Turner syndrome. *SHOX* has been shown to encode a cell type specific transcriptional activator that localizes to the nucleus. Here we report the identification of the Nuclear Localization Signal (NLS) within the homeodomain of SHOX. Deletion mapping identified a non-classical type basic signal, AKCRK, in the recognition helix of the homeodomain. Fusion of this five amino acids stretch to a cytoplasmic reporter protein resulted in its nuclear translocation. During a large scale *SHOX* mutation analysis study, we identified two different mis-sense mutations, R173C (C517T) and A170P (G508C), within the identified SHOX-NLS in three families with Léri-Weill *and* Langer syndrome. Functional analysis of these missense mutations showed that the mutated SHOX protein carrying either of these mutations is unable to translocate to the nucleus. Conversely, we can demonstrate that insertion of the identified signal adjacent to the mutant site can restore its nuclear translocation. Our data explain Léri-Weill and Langer syndrome conditions on a molecular and cellular level and strengthen the growing awareness that mis-regulation of subcellular localization contributes to clinically relevant phenotypes.

C50. Mutations in the human *TBX4* gene cause small patella syndrome

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Small patella syndrome (SPS; MIM 147891) is an autosomal dominant skeletal dysplasia characterized by patellar aplasia or hypoplasia and pelvic anomalies, including disrupted ossification of the ischia and inferior pubic rami. In addition, femur and foot anomalies may accompany SPS. We identified an SPS candidate region of 5.6-cM on chromosome 17q22 by haplotype analysis. Inspection of genes in the SPS critical region revealed the *TBX4* gene as a positional candidate. *TBX4* belongs to the T-box gene family, encoding transcription factors characterized by a strongly conserved DNA-binding motif. Mutations in several T-box genes are associated with human developmental disorders, including *TBX3* mutations in ulnar-mammary syndrome and *TBX5* mutations in Holt-Oram syndrome, both characterized by severe upper limb anomalies. *TBX4* is known to play a crucial role in lower limb development in chicken and mice. In chicken, misexpression of dominant-negative *Tbx4* results in legless phenotypes and in deformed and hypoplastic pelvis. We found putative loss-of-function mutations in *TBX4* in 6 families with SPS, including one nonsense, two missense-, one frameshift- and one splice-site mutation, and skipping of exon 7. Haploinsufficiency appears to be responsible for dominantly inherited SPS in at least some of the families. The phenotype associated with heterogzygous *TBX4* mutations suggest that this gene is involved in late stages of skeletal developmente. The present identification of *TBX4* mutations in SPS patients together with the resembling skeletal phenotype of animals lacking *Tbx4* establish the importance of *TBX4* in the developmental pathways of the patella, pelvis, and feet in humans.

C51. Three candidate genes for autism with a possible role in neuron vesicle trafficking

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Autism is a neurodevelopmental disorder of unknown cause and pathogenesis. To identify candidate genes for autism, we initiated a positional cloning strategy starting from phenotypically normal persons with idiopathic, non-familial autism carrying a *de novo* chromosomal translocation. We identified, in three individuals, three different genes of which the corresponding proteins have a possible function in vesicle trafficking in the brain. Neurobeachin (NBEA) is a neuron-specific A-kinase anchoring protein (AKAP), that recruits the cAMP-dependent protein kinase A (PKA) to endomembranes near the *trans*-Golgi. NBEA is believed to be implicated in neuronal membrane trafficking. The *CLIC4* gene was disrupted in an individual with moderate mental retardation and autism. CLIC4 belongs to the chloride intracellular channel protein family, and has been localized in the perinuclear dense-core vesicles. It may have a role in vesicle acidification. The *Amisyn* gene expression was disrupted in all cells in an individual with a 46,XY t (14;16)(q12;tel). The interpretation of this finding is hampered by a mosaïcism, with loss of the derivative

14 in 30% of the cells. However, Amisyn is thought to be involved in regulating SNARE complex formation, by binding to syntaxin-1 and SNAP25, components of this complex.
Taken together, these findings suggest that vesicle trafficking in neurons may be involved in the pathogenesis in a subset of individuals with autism. The involvement of these three genes in the regulated secretory pathway is currently under study by means of RNAi-mediated gene knock-down.

C52. Functional characterization of mutations found in the PTPN11 gene in patients with Noonan syndrome

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Recent studies have identified missense mutations in the human PTPN11 gene as the cause of Noonan syndrome (NS), a relatively common, but genetically heterogeneous autosomal dominant malformation syndrome. Characteristic features are proportionate short stature, dysmorphic face, and congenital heart defects. PTPN11 encodes the non-receptor protein tyrosine phosphatase SHP-2, which is an important molecule in several intracellular signal transduction pathways that control diverse developmental processes, such as cardiac semilunar valvulogenesis. Almost all mutations identified to date cluster in the amino N-src homology 2 (SH2) domain and the phosphotyrosine phosphatase domain, which are both involved in switching the protein between its inactive and active conformation.
The pathomechanism underlying NS in patients with mutations in SHP-2 protein is presently unknown. Crystal structure analysis of SHP-2 predicted that the mutations would result in a gain of function. To confirm this, we have selected four mutations, including the common Tyr63Cys and Asn308Asp exchanges present in patients with NS. Following cloning of the complete coding sequence of PTPN11, we introduced these mutations in vitro. Subsequently, wild type and mutant SHP-2 proteins were expressed in E. coli and purified. An elisa-based phosphatase assay specific for SHP-2 activity revealed a significantly higher activity of the mutant proteins compared to the wild-type. A similar assay with an auto-activating peptide shall further distinguish between increased basic-activity or auto-activation in the mutants. Our results are an important step towards a better understanding of the pathomechanism of NS.

C53. Functional characterization of wild-type and mutant wolframin, the WFS1 (wolfram syndrome 1) gene product

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Mutations of the WFS1 gene are responsible for Wolfram syndrome, a rare recessive disorder characterized by early-onset, non-autoimmune diabetes mellitus, optic atrophy and further neurological and endocrinological abnormalities. The WFS1 gene encodes wolframin, a novel multispanning membrane glycoprotein of the endoplasmic reticulum. In order to functionally characterize wolframin, we have generated polyclonal antibodies against both hydrophilic termini of the protein. Wolframin was found to be ubiquitously expressed with highest levels in brain, pancreas, heart and insulinoma beta-cell lines. Analysis of the structural features provides experimental evidence that wolframin is embedded in the membrane in an N-cyt/C-lum topology and oligomerizes into higher molecular weight complexes.
In addition, we investigated the molecular mechanisms that cause loss-of-function of wolframin in affected individuals. The effect of different types of WFS1 mutations on wolframin expression and stability was analyzed in mutant cell lines. Most WFS1 mutations investigated here appear to severely affect steady-state levels of wolframin. In patients harboring nonsense mutations complete absence of the mutated wolframin is caused by instability and rapid decay of WSF1 nonsense transcripts. Pulse-chase experiments of mutants expressed in COS-7 cells indicated that also missense mutations of WFS1 lead to instability and strongly reduced half-life of wolframin. Thus, Wolfram syndrome appears to be caused by reduced protein dosage rather than dysfunction of the mutant wolframin.

C54. Loss of desmoplakin isoform I causes severe arrhythmogenic left and right ventricular cardiomyopathy, palmoplantar keratoderma and woolly hair

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Desmosomes are cellular junctions important for intercellular adhesion and anchoring the intermediate filament (IF) cytoskeleton to the cell membrane. Desmoplakin (DSP) is the most abundant desmosomal protein with 2 isoforms produced by alternate splicing. Mutations in desmoplakin have been found to underlie dilated cardiomyopathy associated with skin and hair phenotypes.
This study describes a patient with recessive arrhythmogenic left and right ventricular cardiomyopathy, epidermolytic palmoplantar keratoderma, and woolly hair. The patient showed a severe heart phenotype with an early onset and rapid progression to heart failure. A homozygous stop-mutation, R1267X, was found in exon 23 of the desmoplakin gene in this patient which results in truncation of the larger DSP I isoform. The loss of most of the DSP I specific rod domain and C-terminal was confirmed by western blotting and immunohistochemistry.
These findings are the first to describe a phenotype caused by a mutation affecting only one DSP isoform and they further emphasize the importance of desmoplakin in epidermal and cardiac function. DSP I is reported to be an obligate constituent of desmosomes and the only isoform present in cardiac tissue. This mutation suggests that the cardiac system could develop initially without desmoplakin/desmin IF interactions mediated by the DSP C-terminus. To address this, we have revisited the expression of DSP isoforms in the heart. Preliminary data suggests that specific compartments may express different combinations of isoforms with the absence of DSP II in atria. Our study implicates the importance of the different DSP isoforms during development and cardiac remodelling.

C55. Tiling path resolution mapping of 1p36 deletions by array-CGH: contiguous gene deletion or ‚deletion with positional effect' syndrome?

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The constitutional 1p36 deletion is the most common terminal deletion syndrome, affecting 1 out of 5,000 newborns. It results in the association of a characteristic facial dysmorphy (including: large anterior fontanel, deep-set eyes, flat nasal bridge, asymmetric ears and pointed chin), congenital anomalies and mental retardation. Previous studies suggested that this syndrome was caused by haploinsufficiency of several contiguous genes and proposed potential critical regions for certain clinical findings observed in patients. To further delineate genotype-phenotype correlations in monosomy 1p36, we applied array-CGH using an overlapping clone microarray covering the whole of chromosome 1 at tiling path resolution. Eight patients showing clinical features characteristic of monosomy 1p36 were tested and deletion was confirmed in all cases. Two patients were particularly interesting. The first one had a deletion restricted to the most terminal 2.5 Mb of 1p36.33. The second one had a deletion of 7 Mb in length, starting 3 Mb from the terminal region. Considering that the two patients exhibit very similar features (facial characteristics and mental retardation), the occurrence of non-overlapping 1p36 deletions strongly suggests that monosomy 1p36 may be a 'deletion with positional effect' rather than a 'contiguous gene deletion' syndrome. Altogether, our results indicate that concomitant FISH screening of several 1p36 loci or the use of high resolution array-CGH will be required for full diagnosis of this syndrome.

C56. Non-random asynchronous replication at 22q11.2 and the human 22q11.2 deletion

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The analyses of the replication timing at 22q11.2 were prompted by our finding of a statistically significant bias in the origin of the regions flanking the deletion site in patients with 22q11.2 deletions; the proximal region being in the majority of cases of grandmaternal origin. We hypothesised that asynchronous replication may be involved in the formation of the 22q11.2 deletion, the most frequently occurring interstitial deletion in humans, by favouring the mispairing of low-copy repeats.
Replication timing during S phase at 22q11.2 was investigated by fluorescent in situ hybridisation on interphase nuclei. We report on the detection of non-random asynchronous replication at the human chromosome region 22q11.2, an autosomal locus believed not to contain imprinted genes. Asynchronous replication at 22q11.2 was observed without exception in all 20 tested individuals; these comprised individuals with structurally normal chromosomes 22 (ten cases), individuals with translocations involving the locus 22q11.2 (eight cases) and patients with a 22q11.2 deletion (two cases). The non-random nature of the asynchronous replication was observed in all individuals for whom the chromosomes 22 were distinguishable. The earlier replicating allele was found to be of paternal origin in all cases where the parental origin of the translocation or deletion was known.

C57. Age-related skewing of X chromosome inactivation is not a stochastic process

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A skewed X inactivation may be due to chance, to genetic factors or to a selection against or in favour of cells with a specific genotype. Elderly females have a much higher frequency of a skewed X inactivation pattern than younger females in peripheral blood cells. We have analysed the frequency of skewed X inactivation in females aged 18-101 years, including 200 MZ and 258 DZ twin pairs. A skewed X inactivation was arbitrarily defined as a pattern where 80% or more of the cells have a preferential inactivation of one of the two X chromosomes. The frequency of skewed X inactivation increased from 15% at mean age 55 years to 67% at age 100 years. This increase in skewing continued throughout life, since a lower frequency was found also for females aged 95 years (45%) compared to females aged 101 years (67%). There was no parent-of-origin effect of the preferentially active X chromosome in DZ twins where the paternal X chromosome could be identified. Biometric analysis showed that dominant genetic effects accounted for about 60% of the variance in X inactivation pattern in both young and elderly twins. We found a high intraclass correlation of the X inactivation pattern in the younger MZ twins (0.6), which may in part be due to the shared intrauterine environment for monochorionic MZ twins. However, a similar high correlation was also found in the elderly MZ twins, and indicates that the age-related increase in skewing is not a solely stochastic process.

C58. A new recurrent 8p duplication mediated by an olfactory receptor gene cluster and the MYOM genes: which relationship with the Kabuki syndrome?

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The 8p duplication is a recurrent chromosome anomaly usually associated with the deletion of the distal 8p portion: inv dup(8p). This rearrangement derives from non-allelic homologous recombination between two olfactory receptor (OR) gene clusters (REPP- proximal, REPD-distal: Giglio et al, 2001) resulting in a dicentric chromosome. An asymmetric breakage between the two centromeres of the dicentric results in the formation of the inv dup(8p) that is deleted for the 8p23 portion distal to REPD and duplicated for a region proximal to REPP. Through the molecular definition of two cases of 8p duplication having a phenotype different from that of the inv dup(8p) subjects, we discovered a new recurrent chromosome rearrangement consisting in the interstitial direct duplication of 8p23.1-p23.3. Both cases have the proximal breakpoint at REPP, between clones RP11-303g3/RP11-351i21 and the distal breakpoint between clones RP11-715m14/RP11-1049h7. The distal breakpoint does not contain any OR gene cluster but two inverted copies of another class of repeats in the proximity of the MYOM gene. In both cases the rearrangement occurred at the maternal meiosis. Both patients have mental retardation and dysmorphisms. Although the duplication region of these two patients involves the recently defined Kabuki duplication region (Milunsky, 2003) (10 MB against 3.5 Mb) none of our patients have the Kabuki phenotype. Our findings demonstrate that recurrent chromosome rearrangements could be due to mechanisms other than non-allelic homologous recombination.

C59. Cytogenetic and molecular characterisation of balanced chromosomal rearrangements in patients with limb malformations and breakpoints in chromosome band 2q31 near the HOXD complex

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Characterisation of disease-associated balanced chromosome rearrangements has proven to be a promising starting point in the search for candidate genes and regulatory elements. We have identified and investigated 3 patients with limb abnormalities and chromosomal breakpoints in band 2q31. In patient 1, the balanced t(2;10)(q31.1;q26.3) translocation is associated with skeletal malformations including severe brachydactyly and syndactyly. Other clinical features present in this patient include mental retardation, hypoplasia of the cerebellum, scoliosis, and ectopic anus. Patient 2, who carries a similar t(2;10)(q31.1;q23.33) translocation, shows hypoplasia of the ulna only. Patient 3 carries a pericentric inversion of chromosome 2, inv(2)(p15q31). Her phenotype is characterized by bilateral aplasia of the fibula and the radius, bilateral hypoplasia of the ulna, unossified carpals bones, and hypoplasia and dislocation of both tibiae.
By fluorescence *in situ* hybridisations, we have mapped the breakpoints to intervals of approximately 100 kb. Additionally, we have cloned the breakpoint of patient 1. Computational analysis of the breakpoint regions revealed that all breakpoints in 2q31 map in the vicinity of the homeodomain transcription factor D (HOXD) cluster, known to play important functions in limb patterning and growth, and provided no indication for a disrupted gene. HoxD gene expression in the mouse has been shown to be regulated by cis-acting DNA elements acting over distances of several hundred kilobases. Therefore we suggest that in all 3 reported cases the rearrangement events most likely exerted deleterious effects by disturbing normal *HOXD* gene regulation.

C60. 22q11.2 microdeletion Syndrome: small deletions (1.5-2 Mb) are more frequent in familial cases than in sporadic.

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22q11.2 microdeletion Syndrome has a frequency in the general population of 1:4000-6000 newborns. Molecular studies have

revealed a common large deletion (3 Mb) in 90% of the cases, a common small deletion (1.5-2 Mb) in 8%, and atypical deletions in 2%.
FISH studies were done using D22S75 and TUPLE1 locus-specific probes. Molecular analyses were carried out by PCR-amplification of 12 to 16 polymorphic markers of 22q11.2 region. By FISH, 59 patients were found to have the microdeletion 22q, and 6 of them (10%) were familial cases. Five of these families were subjected to molecular studies, together with their relatives (19 individuals in total). Cytogenetic and molecular results confirmed in all cases a maternal inheritance of the deletion. In a family with three affected generations, the deletion was transmitted by the proband's maternal grandfather. By microsatellite genotyping, we identified 1 family with the common large deletion (3 Mb) and 3 families with the common small deletion (1.5-2 Mb). In another family, the lack of parental data didn't allow the deletion size to be determined.
These results suggest a common small deletion predominance in 3/4 familial cases (75% vs. 8% reported in the general deleted population). This supports the hypothesis of a bigger tolerance for small deletions in inherited cases. Thus we recommend to check for a possible familial aggregation of the syndrome, when a common small deletion (1.5-2 Mb) or an atypical deletion is found.

C61. Mutations in the gene encoding the cytoskeletal protein filamin B affect skeletogenesis, vertebral segmentation and joint formation in humans.

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The filamins (encoded by the genes *FLNA, FLNB* and *FLNC*) are cytoplasmic actin cross-linking proteins that regulate cell shape and migration. They link transmembrane proteins to the cytoskeletal actin network and modulate intracellular signaling and protein trafficking. Using positional mapping/candidate gene screening and direct candidate gene mutation analysis, mutations were identified in *FLNB* underlying four human skeletal disorders. Homozygosity or compound heterozygosity for stop codon mutations were found to underlie autosomal recessive spondylocarpotarsal syndrome, a disorder characterized by vertebral malsegmentation and carpal and tarsal bone coalition. Missense mutations were associated with autosomal dominant Larsen syndrome, a phenotype characterized by multiple large joint dislocations with vertebral and craniofacial anomalies, and in the perinatal lethal atelosteogenesis I and III phenotypes, which feature widespread and severe defects of skeletal development and joint formation. Immunohistochemical and immunoflourescence studies show that filamin B is expressed in growth plate chondrocytes and in the developing vertebral bodies. Together these data reveal an unexpected role in vertebral segmentation, joint formation, and endochondral ossification for *FLNB.* Filamin B becomes the second member of the filamin family to be shown to mediate critical functions in morphogenesis in humans, especially those that relate directly to skeletogenesis. The molecular pathology of disorders caused by mutations in genes encoding filamins suggests a novel and essential role for cytoskeletal architecture in skeletogenesis.

C62. Acromesomelic chondrodysplasia with genital anomalies due to a homozygous mutation in *BmpR1b*

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We report on a 16 year old Turkish girl, offspring of consanguineous parents, who presented with a severe form of limb shortening, hand/foot malformation, and infertility. The limb deformities showed an increasing severity in a proximo-distal gradient, fibula aplasia, severe brachydactyly with absent phalanges as well as fusion and deformation of the carpal/tarsal bones. These features resembled the phenotype spectrum of Grebe syndrome, Hunter Thompson syndrome and DuPan syndrome, disorders which are due to homozygous mutations in the *Growth Differentiation Factor 5* (*GDF5*). Beside the typical features of *GDF5*-related syndromes, hypoplasia of the uterus and absence of the ovaries were additionally demonstrated on the pelvic ultrasound study. The hormonal status of the patient was consistent with hypergonadotropic hypogonadism.
Sequencing of *GDF5* revealed no mutation; thus a mutation analysis in *Bone morphogenetic protein Receptor 1b* (*BmpR1b*), which is a receptor for GDF5, was performed. A homozygous 8 bp deletion (del376-383) was detected in *BmpR1b*.
This is the first description of acromesomelic chondrodysplasia and infertility in a patient caused by a homozygous mutation in *BmpR1b*.

C63. A novel X-linked leukodystrophy with metaphyseal chondrodysplasia maps to Xq25-q27

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Leukodystrophies are progressive diseases characterized by defective central nervous system myelination. Here we report on a family with four affected males in three generations. All patients presented with a unique phenotype consisting of symptoms of a slowly progressive leukodystrophy and that of a metaphyseal chondrodysplasia. The pedigree is strongly suggestive for an X-linked recessive trait. First symptoms (tremor, spacticity, ataxia, and walking difficulties) were usually noted in early childhood. Physical examination of the two living patients, now 15 and 17 years old, showed various dysmorphic features such as hypertelorism and broad, depressed nasal bridge with anteverted nose. The patients were severely handicapped from a progressive visual impairment and mental deterioration. MRI showed a severe leukodystrophy with almost complete loss of white matter. X-ray analysis of the joints demonstrated metaphyseal alterations with flaring. Both a maternal uncle and great-uncle of the patients died of a debilitating neurological disorder at the age of 36 and 26 years, respectively.
A gross structural aberration of the X chromosome was excluded. Two-point linkage analysis yielded positive lod scores for a total of 5 loci on Xq with maximum z values of 1.31, 1.41, and 1.62 at theta=0.00 for DXS1047, DXS6855, and HPRT, respectively. Analysis of individual recombinants mapped the disease locus between DXS8093 and DXS1232 in Xq25-q27 and defined a region of approximately 14 Mb that harbours the putative disease gene.

C64. Functional analysis of the receptor tyrosine kinase Ror2 in mouse and chick

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Truncation mutations of the orphan receptor tyrosine kinase ROR2 cause dominant brachydactyly type B (BDB) which is characterized by hypoplasia or aplasia of distal phalanges and nails. Recessive Robinow syndrome (RRS), exhibiting a different phenotype, was shown to be allelic to BDB. While RRS is likely to be caused by loss of function mutations in ROR2, the functional consequences

of the mutations in BDB remain elusive. To characterize the Role of Ror2 in cartilage condensation and growth, we took different in vitro and in vivo approaches. Yeast two-hybrid screening provided novel intracellular association partners linked to receptor signaling and signal modulation. Additionally, we performed Affymetrix chip hybridization with RNA from Ror2-/- and wildtype mice to identify downstream targets of Ror2. Automated in-situ hybridization of the candidate genes on mouse limbs indicates a high number of genes co-expressed with or expressed adjacent to Ror2. In order to analyze the consequences of BDB mutations in vivo, we overexpressed mutant constructs in chicken embryonic limbs using a retroviral system. Unexpectedly, the overexpression did not result in a brachydactyly phenotype, but in a severe growth retardation of all cartilage elements accompanied by a deranged growth plate architecture. Similarly, expression of Ror2 truncated at a position comparable to human BDB in a knock-in mouse (De Chiara et al. 2000) was reported to result in loss of middle rather than terminal phalanges. This points towards remarkable differences in the molecular mechanisms underlying digit formation and especially differentiation of the terminal phalanx in human, mouse and chick.

C65. Hereditary nanism with autosomal recessive inheritance in Yakut population (Russia)

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The Yakuts (Sakha) is the nation, which is populated in the Republic of Sakha (Yakutia)(Siberia,Russia). One of the most frequent reasons of referring for medical-genetic consultation in the region is the growth retardation of children. One form of autosomal recessive nanism among the Yakuts was observed. 40 patients from 34 families, including 22 female and 18 male, from 0 till 57 years were examined during this observation. This syndrome is characterized by prenatal growth failure (weight at birth - 2300 g., growth - 42 cm). Intelligence is normal. The average height of the patients by the age of 3 is 75 cm and weight is 9,75 kg, the average height by age of 7 is 96,1 cm and weight -13,9 kg, by the age of 11 - 115,5 cm and weight -25,0 kg. The height of the patients at the age of 18 and older is about 133-138 cm. Facial dysmorthism reminiscent of Three M syndrome. Clinical features include short proportional stature, pre and post-natal growth failure, hydrocephaloid skull, triangular face, epicanthus, hypoplasia of cheekbones, short neck, short and wide thorax with deformation, muscle hypotonia, lordosis, huge stomach, brachydactylia, flat-footedness and ledgy heels. Sexual development is normal. Laboratory researches of a mineral exchange are without any pathology, thyroid and growth hormone concentration is normal. The bone age corresponds to passport age. Children usually enter the school with one-year delay because of growth delay and study in general schools. The patients with very low height experience difficulties in social adaptation.

C66. The European Skeletal Dysplasia Network (ESDN) [www.esdn.org contact: info@esdn.org]

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The European Skeletal Dysplasia Network (ESDN) is a pan-European referral and communication network, which allows access to diagnostic and research services from any country within the European Community. The ESDN has been established for two years and is funded by the European Union and the Swiss Government*.
The aims of the ESDN are two-fold:
1. To identify the cellular, molecular and genetic factors that cause skeletal dysplasias.
2. To develop effective approaches to diagnose skeletal dysplasias.
The ESDN provides a diagnostic service which includes clinical review of all skeletal dysplasias and the molecular analysis of 21 genes associated with more than 28 skeletal dysplasias. In 2002, the ESDN received approximately 500 patient referrals from 17 European countries. 290 diagnostic tests were performed and causative mutations were identified in 120 patients. In 2003, these figures increased to 527 referrals from 22 different European countries, 359 tests performed and mutations identified in 193 patients.
The ESDN is continuing to develop their diagnostic referral and quality systems and in February 2004, the ESDN case management web-portal was launched. This secure electronic system allows the ESDN to manage the diagnostic network to a high level of efficiency whilst at all times protecting the confidentiality of patient referrals and ensuring that adequate consent procedures are in place. All information about the ESDN and access to the case manager can be found at www.esdn.org. Ultimately, this integrated and multidisciplinary approach will promote the correct diagnosis of many skeletal dysplasias.
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C67. Fast estimation of critical values of correlated genome scans for linkage

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Estimates of study specific critical values for linkage scans (suggestive and significant thresholds) are important to identify promising regions. Often, scientists must perform multipoint linkage scans for several correlated traits or the same trait under different statistical models. Consequently, the outcome consists of several correlated multipoint linkage trace and many researchers chose to present the signal peaks from all traces, i.e. they report the performance of the pointwise maximal linkage trace. In this paper I propose a fast and concrete recipe for finding study specific critical values for the maximal linkage trace. Critical values could be derived theoretically or empirically. Theoretically-derived values are often conservative due to their assumption of fully informative transmissions. Empirically-derived values are computer and skill intensive and may not even be computationally feasible for large pedigrees. I propose a method to estimate critical values for correlated multipoint linkage traces using standard, widely used statistical software. The proposed method estimates study-specific critical values by using autoregressive models to estimate the correlation between standard normal statistics at adjacent map points for each trace and then use it, along with cross correlation with the other traces, to estimate study-specific critical values. The method is evaluated using different family structures and density of markers, under both the null hypothesis of no linkage and the alternative hypothesis of linkage between marker and disease locus. Overall, the proposed method appears very accurate in predicting critical values especially when the AR models are applied to the differenced time series.

C68. Accounting for strong age-specific sex-limitation in IgE QTL linkage analysis

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We have measured levels of total serum immunoglobulin E (IgE) and other asthma related phenotypes in 934 sib-pairs from 803 Australian twin families ascertained via one asthmatic twin proband. A genome scan (~10cM) was performed with subsequent fine mapping (1-5cM) of candidate regions. Overall, the sib correlation for logIgE was 0.25, with no significant differences between same-sex (SS) and opposite-sex (OS) sib pairs, nor between young (both sibs ≤20) or older sib-pairs. In spite of this apparent covariance homogeneity, a strong age-specific sex-limitation was found: young OS sib-pairs had an IgE correlation (r=0.04, 95% c.i.: -0.12-0.20) significantly lower than young SS pairs (r=0.35, 95% c.i.: 0.21-0.47). This sex-specific effect was no longer observed at a later age, with old OS pairs having a logIgE correlation (r=0.23, 95% c.i.: 0.09-0.36) similar to older SS pairs (r=0.21, 95% c.i.: 0.08-0.33). Thus, young OS sib-pairs

displayed a degree of atopic discordance larger than expected from their degree of relatedness. If unaccounted for, this can significantly reduce the power of linkage analysis under the hypothesis of linkage. A similar effect was observed for the skin-prick test data. Since ~25% of our sib-pairs consist of OS pairs with at least one young sib, this sample provides appropriate data to compare the power of linkage analysis under different genetic models, namely sex-specific age-of-onset, classical sex-limitation and parental imprinting. Fine mapping data from chromosome 11 will be used to test these models, namely at the known asthma gene FCER1B and near the candidate genes ELF5 and EHF.

C69. Identification of genes linked to complex disease

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We have developed a novel technology for gene identification in complex disease called GenomeHIP® which overcomes many of the limitations of more traditional linkage mapping methods. GenomeHIP compares the genomes of pairs of related individuals with the same disease and eliminates the DNA segments that are not identical between the individuals. The GenomeHIP regions are mapped by hybridisation of the DNA segments that are identical between the two related individuals onto a BAC microarray representing the human genome at a 1 Mb resolution. Analysis of the microarray data allows the identification of short regions, spanning on average 2 Mb, that are shared between the different pairs of related individuals. As the individuals share the same disease, these regions will most likely contain the disease-linked genes.
GenomeHIP was applied to 167 sib pairs affected with obesity and 116 sib pairs affected with autism. This allowed the identification of six narrow regions linked to obesity. Analysis of three of the loci revealed a G-protein coupled receptor, involved in energy metabolism, to be associated with obesity. The association was supported by functional data showing a loss of function of the receptor expressing the associated haplotype. Two other genes active in the same pathway also appear to be associated with obesity. At least five statistically significant loci with strong candidate genes have been identified in autism. One new synaptic modulator gene has already been confirmed in association studies.

C70. Dissecting the ApoE locus with respect to Alzheimer‘s disease using novel association statistics

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A major challenge in genetic studies of complex traits is the characterization of small genetic effects at potentially many loci. Alzheimer‘s disease is one of few examples where a predisposing causal effect of an allele (ApoE\epsilon_4) could be replicated in numerous samples. The ApoE locus can therefore be used to evaluate statistical procedures with respect to their capability of detecting a causal locus and exploring further information like haplotype frequencies or penetrance parameters. We apply novel statistical procedures to a data set of Alzheimer‘s disease containing nuclear families (Martin et al., AJHG 2000). Using likelihood based methods we show, that haplotype frequencies in the region can be estimated consistently even if the causal locus is unobserved, i.e. linkage disequilibrium (LD) of observed haplotypes with the unobserved causal locus can be reconstructed. Furthermore, the effect of unlinked, unobserved loci which also influence the phenotype can be estimated. It is therefore possible to assess the relative contribution of the ApoE\epsilon_4 allele with respect to Alzheimer‘s disease, even if it is not observed. We also investigate nonparametric methods to test contributions of loci with respect to phenotypes. These methods are more robust since they are based on fewer assumptions about the genetic situation. As shown by simulation they might be less powerful than the LR-tests and provide less information about parameters of interest.

C71. Effects of population substructure on association studies: Calpain-10 in patients with diabetic nephropathy

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Type 2 diabetes and diabetic nephropathy are a result of an interaction between genetic and non-genetic factors. A range of genome-wide scans has been performed in order to identify diabetes susceptibility loci. In a Mexican-American population a diabetes susceptibility region was found on chromosome 2q37.1. The responsible haplo(geno)type was identified and consisted of SNPs UCSNP-43, -19, -63 within intron 3 of the calpain-10 gene. We tested whether our two groups of type 2 diabetic patients on dialysis and healthy controls (German ancestry) contained individuals interfering with the results by a hidden diverse ethnic origin. We identified four different “genetically comparable subsets” of cases and controls (group 1: n=547 cases/101 controls, group 2: n=39/13, group 3: n=26/13, group 4: 0/87). We tested the three UCSNPs and any possible haplogenotype for association with type 2 diabetes in the total groups and in the identified subgroups of cases and controls. The analyses in all individuals revealed no evidence for association. However, we found association of the common allele 1 of UCSNP-63 in group 1 (P=0.002) when analyzing the groups separately. In this group the haplotype 112 occurred more often in cases than controls (P=0.006). This effect was due to haplogenotype 112/121 (OR=0.27, 95% CI=0.13-0.57). Our results indicate that testing for population substructure in an apparently homogeneous population can severely affect the outcome of association analyses. We propose our procedure for any genome scan, based on linkage or association, in order to increase the chances for the identification of causative sequence variants.

C72. Disregulation of multiple LOX-1/OLR1 mRNA isoforms as risk factor in myocardial infarction.

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Myocardial infarction (MI) is a significant cause of mortality. Substantial data support a role for oxidized LDL (oxLDL) in the etiology of MI. The human *LOX-1/OLR1* gene encodes the endothelium-derived lectin-like oxidized-LDL receptor, which is involved in binding, internalization, and proteolytic degradation of oxLDL, suggesting that it may play significant role in atherogenesis/MI. We previous identified a group of SNPs contained in a specific disequilibrium block within this gene that are associated with the risk to develop coronary atherosclerosis (CAD)/MI (Mango et al. J Med Genet 2003 Dec;40(12):933-6.). As the disease-associated SNPs identified did not affect the *LOX-1/OLR1* aminoacid sequence, we investigated their functional effect . Using isolated mononuclear cells from randomly selected patients, we promoted their transition to mature dendritic cells by stimulation with oxLDL . We were able to demonstrate the existence of multiple LOX-1/OLR1 transcripts. One of these forms, flOLR1 corresponded to the full length transcript, while the other, named “Loxin”, was lacking exon 5. To quantify each isoforms, an isoform-specific kinetic RT-PCR analysis was carried out. A significant difference between gene expression and genotype was observed. We found that the ratio of loxin/flOLR1 mRNA in dendritic cells was significantly lower in IVS4-73 T/T-positive disease-susceptible individuals compared to C/C protected individuals (P=0.001). Consistent with a semi-dominant mode of inheritance of the disease locus at LOX-1/OLR1, the heterozygous genotype expressed more loxin mRNA that the homozygous susceptible genotype (P=0.03). Our results suggest that the 1.1 Kb region, containing the linkage disequilibrium block, determines the efficiency of the splicing.

ESHG Posters

P0001. Selective antibody immune deficiency in a patient with Smith-Lemli-Opitz syndrome

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Smith-Lemli-Opitz syndrome is a rare autosomal recessive disorder characterized by multiple congenital anomalies and various degrees of cognitive deficits. This condition results from deficiency of 7-dehydrocholesterol reductase, a critical step in cholesterol biosynthesis. Children with Smith-Lemli-Opitz syndrome have frequent infections, particularly of the respiratory tract. Immunodeficiency, however, is not recognized as a part of this metabolic condition. Frequent infections are usually attributed to a decreased patient mobility and reduced respiratory effort secondary to muscular hypotonia and mental retardation, which are often present in affected individuals. We describe a patient with Smith-Lemli-Opitz syndrome and recurrent respiratory infections who was found to have a variant of common variable immune deficiency, selective antibody deficiency. The immunologic diagnosis was based on absent immune response to 12 pneumococcal serotypes and hepatitis B vaccine and on the inability to break down red cells with isoagglutinin B. Therapy with intravenous IgG was initiated. Infections were less severe, although still occurred in the similar frequency after initiation of the IVIG therapy. This finding prompts the need for a higher index of suspicion for an underlying immunodeficiency in patients with Smith-Lemli-Opitz syndrome who present with recurrent and chronic infections. Early recognition and appropriate therapeutic interventions may decrease severity of infections, prevent potentially fatal infections and eventually improve the quality of life in these patients.

P0002. Detection of novel notch3 mutations by DGGE and direct sequencing in patients with CADASIL

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CADASIL syndrome (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a hereditary autosomal disorder which is caused by mutations in the notch3 gene. The disease is characterized by recurrent ischemic episodes, migraine with aura, progressive subcortical dementia and early onset strokes.
Due to clustering of notch3 mutations in exon3 and 4 screening protocols mainly focused on detection of missense mutation in these exons. Therefore standard procedures miss 30-40 % of notch3 mutations . To achieve higher sensitivity we established a DGGE-screening protocol for detection of mutations in all exons of the notch3 gene.
Up to now we were able to detect 13 mutations/polymorphisms in our ongoing screening. One mutation (C402Y) was a novel nucleotide change leading to a cysteine to tryptophan amino acid change in the EGF-like domain. In one family, we identified a novel cysteine to serine mutation (C449S).
Additionally, we found a novel splice site mutation (IVS6ds+5G>A) in a 59 year old patient who was originally thought to present with multiple sclerosis. A skin biopsy was tested positive for CADASIL. So far, this is the second report of a disease-causing splice site mutation of the human notch3-gen.
Our preliminary data indicate that DGGE is a highly sensitive screening method for CADASIL diagnosis. Our results underline the importance of a screening program, which is capable to detect notch3 mutations in all exons.

P0003. Androgen insensitiviy syndrome

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The androgen insensitivity syndrome (AIS) is the single most common cause of male pseduohermaphroditism, i.e., deficient masculinization which is not explained by disturbed testis development. In its most severe form it affects at least 1/20 000 newborn 46XY males. This X-linked recessive disorder is caused by mutations in the androgen receptor (AR) gene, and it has classically been divided into two subgroups according to severity; complete (CAIS) and partial androgen insensitivity syndrome (PAIS). In the complete form, the 46XY individual presents as a phenotypically normal girl, except for absence of sexual hair. These patients have intra abdominal testes and , due to regression of mullerian ducts, a short vagina, no uterus and lack oviduct. Partial forms of AIS present as varying degrees of under masculinization, ranging from a predominantly female phenotype to boys with genital malformations, such as hypospadias or cryptorchidism. It has also been speculated, that subtle androgen receptor defect could cause impaired spermatogenesis without genital malformations.
Two Hundred patients from 65 families were examined . All patients were examined clinically and classified according to the degree of symptoms. In 49% of the patients several chromosomal abberations were identified. In addition ten missense mutations are described in the AR gene of patients phenotypically ranging from complete androgen insensitivity to men with preserved fertility. Mean age of referral patients is around 17 years which causes a lot of pressure both on councellor and also patients and their families for accepting the test results and living new condition.

P0004. Dominantly inherited progressive pseudorheumatoid dysplasia with hypoplastic metatarsals

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We present four related patients with **P**rogressive **Ps**eudo**r**heumatoid **D**ysplasia(PPsRD) each with distinctive history, unique phenotype and some peculiar radiographic findings. The history was characterised by weather dependent articular pain. The unique phenotypic features were hypoplasia/dysplasia of one or two toes. Peculiar radiographic findings were hypoplasia of 3rd and 4th metatarsals, platyspondyly with rectangular shape of the lumbar spinal canal, progressive narrowing of the joint spaces and early synovial chondromatosis. Finally, the condition was inherited as a dominant trait. This constellation of abnormalities constitutes a distinct form of PPsRD. PPsRD must be differenciated from other bone dysplasias specifically spondyloepiphyseal dysplasias, autosomal dominant spondylarthropathy, juvenile rheumatoid arthritis and osteoarthritis.

P0005. Familial hypodontia associated with supernumerary teeth: a genetic link?

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Congenitally missing teeth and teeth formed in excess are opposite tooth number anomalies. A concomitant occurence of hypodontia and hyperdontia in the same dentition is a quite rare dental phenotype in man. As an isolated trait, both hypodontia and supernumerary teeth have a genetic basis, but can also be caused by disturbances during tooth development. The genetic etiology of concomitant hypodontia and hyperdontia generates much debate. The aim of our study was to investigate the genetic cause of hypodontia associated with “extra” teeth because a genetic link was suspected. In a sample of 76 patients with familial hypodontia we analyzed dental phenotype and the inheritance pattern of the condition. Three cases were selected based on their distinct clinical phenotypic association: simultaneous presence of hypodontia and supernumerary teeth in the permanent dentition. Intraoral and radiographic examinations were performed. Index cases have shown variability in expression of clinical manifestations including the type, number and location of affected teeth. Family studies of the index cases have shown an autosomal-dominant manner of inheritance with lack of penetrance and variable expression for hypodontia. Only index cases expressed both tooth number defects.Our data suggested that hypodontia is aetiologically distinct from supernumerary teeth and the dental anomalies association is coincidental. More cases showing similar phenotypes will be required to permit definitive conclusions.

P0006. Four potassium channel mutations account for 73% of the genetic spectrum underlying long QT syndrome (LQTS) and provide evidence for a strong founder effect in Finland

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Long QT syndrome (LQTS) is an inherited arrhythmic disorder characterized by a prolonged QT interval on ECG, ventricular arrhythmias and risk of sudden death. Mutations in five cardiac voltage-gated ion channel genes, including KCNQ1, HERG, SCN5A, KCNE1 and KCNE2, constitute the principal cause of this disorder. Typically, each family carries its own private mutation, and the disease manifests with varying phenotype and incomplete penetrance, even within particular families. We had previously identified 14 different LQTS-causing mutations in 92 Finnish families. In the present study, we conducted a systematic search for mutations in the five LQTS genes among 188 additional unrelated probands. The screening was performed by denaturing high-performance liquid chromatography (dHPLC) and DNA sequencing. Nineteen novel and 12 previously described mutations were identified. Collectively, these data extend the number of molecularly defined affected Finnish LQTS families and patients at present to 150 and 939, respectively. Four presumable founder mutations (KCNQ1 G589D and IVS7-2A>G, HERG R176W and L552S) together account for as much as 73% of all established Finnish LQTS cases. In conclusion, the extent of genetic homogeneity underlying LQTS in Finland is unique in the whole world, providing a major advantage for screening and presymptomatic diagnosis of LQTS, and constituting an excellent basis to study the role of genetic and nongenetic factors influencing phenotypic variability in this disease.

P0007. 22 Cases with Williams Beuren Syndrome : Clinical findings and management guidelines

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Williams Beuren Syndrome is characterized by typical facial appearance (elfin face), cardiac malformations, mental retardation and infantile hypercalcaemia caused by submicroscopic chromosome deletion that includes the elastin gene at 7q 11.23. Clinical and laboratory findings of 22 patients with Williams Beuren Syndrome which were followed at Hacettepe University Dept. of Pediatrics Genetic Unit between 1983 and 2003, were reviewed. Characterized facial findings and developmental delay were found at 96%, mental retardation and dental anomalies at 86%, behavioral disorders at 77%, cardiac malformations (supravalvular aortic stenosis was the most common) at 41%, conductive hearing loss at 33%, central nervous system malformations at 31%, infantile hypercalcaemia at 4% of the patients. Deletion of 7q11.23 region was detected at all of the nine patients whom analysis of FISH had been performed. Evaluation of dental problems of patients showed the most common features are malocculusion size and shape anomalies. Also in one patient a talon cusp was observed in the mandibular incisor tooth. Early recognition of Williams Beuren Syndrome is very difficult because development of clinical findings is progressive. Understanding of genotypes-phenotypes relationship of Williams Beuren Syndrome will supply new methods of early diagnosis and pre / postnatal treatment. We have reviewed the published literature and reevaluated the diagnostic and management guidelines of this syndrome. We conclude that clinical use of these guidelines is important both for the treatment and rehabilitation of the current problems and preventing of the complications.

P0008. Williams-Beuren Syndrome and Congenital Pulmonary Venolobar Syndrome: A Coincidental Finding or True Association?

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Williams-Beuren syndrome (WBS) is a relatively common genetic disorder characterized by distinctive facial appearance, vascular stenoses, growth retardation, mild mental deficiency, characteristic personality profile, and some other physical and behavioral abnormalities. It occurs in ~1 of every 20,000 births. We herein report on one 4-year-10-month-old male patient with this syndrome, which's confirmed by FISH study using the digoxigenin-labeled Oncor WBSCR probe containing the elastin gene. He also has a very rare pulmonary anomaly, i.e. bilateral intralobar pulmonary sequestration and horseshoe lung which is correlated with congenital pulmonary venolobar syndrome (CPVS). We raise the question of the relation between WBS and CPVS, and suggest a possible association between the two. There are at least 17 genes identified in the WBSCR and neighboring region at 17q11.2, most of them are not clarified with their functions. The report of this case brings up an additional argument for related vascular and bronchopulmonary abnormalities or interaction with growth defect in the pathogenesis of WBS.

P0009. X-linked Dominant Chondrodysplasia with hydrocephaly

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We describe a family with an X-linked dominant chondrodysplasia. Four males and six females are affected through four generations. Identification of skeletal anomalies and hydrocephaly during the pregnancy of three male fetuses led to termination of the pregnancies. A fourth affected male died at 6 days of life. The four children had chondrodysplasia, hydrocephaly and facial dysmorphism with microphthalmia. X-rays showed severe platyspondyly and various bone anomalies with a particular cupuliform shape of the phalanges. The carrier females are less affected and have a small stature, sometimes associated with hemihypertrophy and mild mental retardation. This condition appears to represent a new X-linked dominant chondrodysplasia.

P0010. Tuberous sclerosis in large pedigree with variable expression

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A25 years old woman was referred to genetic clinic because of a skin disorder. She had hypomelanic macules in abdomen and lumbar region, a small angifibroma, a skin tag on the back of the neck and axillary. In addition she had psychological and behavioral problems. These symptoms had first been noted at 12 years of age. A skin biopsy confirmed the diagnosis of T.S. Drawing of the pedigree demonstrated that another 20 cases were present in the family with a variable degree of skin abnormality, epilepsy, depression, suicide, clonic seizures, shagreen patch, behavorial and psychological problems, mental retardation and learning disability. T.S is inherited as an autosomal dominant with variable expression and locus heterogeneity. There are major and minor features in T.S. Major features are facial angiofibroma or forehead plaque, hypomelanic macules, shagreen patch, subependimal nodules, and epilepsy. The most important minor feature is dental pits. Approximately 60-70% of cases are due to new mutation. In our family, the origin of the mutation was in one of the mothers grandparents. Mutations in two genes, TSC1 and TSC2 are responsible for 70% cases of T.S.

P0011. Gapo Syndrome:first Report Of Two Cases In Iran

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GAPO syndrome is a rare autosomal recessive genetic disorder. The primary signs of this syndrome are as follows: Growth retardation, Alopecia totalis, Pseudodentia and Optic atrophy. GAPO syndrome was first reported in 1947 and 24 cases have been diagnosed by the year 2000. Here we report a 21 year-old male with additional signs including peculiar facial appearance, proportionate short stature,

frontal bossing, keratoconus, prominent eyes, anteverted nares, prominent /everted lower lip, prominent upper lip etc. The parents of the reported case are first cousins and they have another daughter with this syndrome in their family.

P0012. Trigonencephaly, Variable Limb Anomalies, Cutis Laxa: A new Autosomal Recessive Syndrome?

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Here we report two Saudi Arab brothers born to a phenotypically normal first cousins couple. Both displayed a very characteristic clinical manifestations including trigonencephaly caused by premature closure of the metapoic suture, narrowing of coronal sutures, variable limb defects (cutaneous syndactyly, pre-axial polydactyly, and brachydactyly), cutis laxa, and developmental delay. The karyotype on the amniotic fluid on the first child showed a balanced reciprocal (46, XY,t(2;6)(p15;q23). However, the second child's karyotype was normal on the amniotic fluid. The parent's chromosomal study on the blood lymphocytes was normal. We believe the constellation of anomalies in this family most likely represent a new autosomal recessive disorder.

P0013. Binder syndrome associated with maternal biliary lithiasis in early pregnancy: a possible role for vitamin K deficiency.

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Binder syndrome is a maxillonasal dysplasia characterized by midfacial hypoplasia, lack of anterior nasal spine, and malocclusion, associated in some patients with short terminal phalanges of fingers and toes and radiologic features of chondrodysplasia punctata. It has been suggested *(Howe et al, Am J Med Genet. 1995;58:238-44, Am J Med Genet. 1997;71:391-6)* that vitamin K-deficiency during human pregnancy can be caused by the therapeutic use of warfarin or phenytoin, causing abnormal development of the cartilaginous nasal septum. It was proposed (*Howe et al, Teratology. 1992;46:379-90*), on the basis of a rat model, that the septal growth retardation occurs because the drug-induced extrahepatic vitamin K deficiency prevents the normal formation of the vitamin K-dependent protein (Bone gla Protein) in the embryo. We report what is, to our knowledge, the first published observation of a child with facial and distal limb characteristics of Binder syndrome, whose mother was operated upon for biliary lithiasis in early pregnancy. Because of hyperemesis this mother was hospitalized at 13 weeks of pregnancy and a biological screening revealed a prothrombin time at 27%, a decrease in clotting factors II, VII, and X, while factor V was at 162%. These are indications of vitamin K deficiency. Abdominal ultrasonography revealed several biliary lithiases and the mother was operated at 18 weeks of pregnancy. We suggest that this biliary lithiasis-induced vitamin K deficiency, like anticonvulsant- or warfarin-induced vitamin K deficiency might have caused abnormal development of the cartilaginous nasal septum and distal phalanges in this child.

P0014. Genetic counselling of the family with a proband with Beals syndrome

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Congenital contractural arachnodactyly (CCA, Beals syndrome, MIM121050) is a rare disease with autosomal dominant type of inheritance. The proband with CCA and his parents are under our observation. Proband was born after the first pregnancy to a 21-yr-old female and 25-yr-old male by spontaneous vaginal delivery. His parents are nonconsanguineous. This pregnancy was complicated with early and late toxemia and direct contact with chickenpox patient at 8 weeks. Proband birth weight was 2850 (25centile). His length was 50cm (25centile). Multiple dysmorphic features were observed at birth. Proband aged about 6 had some distinctive features that were typical for Beals syndrome: his length was 127cm (97centile), his weight was 22.5kg (50centile), Marfanoid habitus, frontal bossing, small mandible, crumpled ears, high-arched palate, pectus carinatum, arachnodactyly and camptodactyly, flexion contractures of proximal interphalangeal joints and multiple joint contractures. Spina bifida posterior displastica S1 and spina bifida posterior occulta L5 were shown on X-ray films. Orthopedics operations were accomplished at the proband age of 9mo, 3yr, 4yr and 5.5yr. Our proband and his father are very much alike, but father has milder phenotype: Marfanoid habitus, frontal bossing, crumpled ears, myopia (-4), mild flexion contractures of proximal interphalangeal joints. We suppose to diagnose Beals syndrome for our proband and his father. It is very important to have a chance of molecular investigation for correct diagnosis and genetic counseling.

P0015. Hereditary deafness and palmoplantar keratoderma associated to the A7445G mtDNA mutation in a Portuguese family

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Over the last several years, some forms of sensorineural hearing loss have been found to be associated with mutations in mitochondrial DNA (mtDNA). However, dermatoses associated to mitochondrial mutations are rare. Non-epidermolytic palmoplantar keratoderma (NEPPK) refers to a keratinization disorder characterized by hyperkeratosis on the palms and soles without epidermolysis. Mitochondrial DNA A7445G point mutation in the tRNASer(UCN) gene has been shown to be responsible for familial NEPPK associated to deafness, without any additional features.To date, just a few cases have been described.
We report a Portuguese pedigree presenting an inherited combination of NEPPK and sensorineural deafness compatible with maternal transmission. Clinical expression and age of onset of NEPPK and deafness were variable. All affected members have first been screened for *GJB2* mutations with negative results. Molecular analysis then focused on mtDNA A7445G point mutation. Screening for this mutation was performed by amplifying a 215 bp fragment followed by digestion with *Xba*I. The amplified fragment looses a *Xba*I restriction site in the presence of A7445G mutation, therefore only DNA not containing the mutation is cleaved. Restriction analysis showed that *Xba*I site was absent in all the affected members of the family revealing the presence of the mutation in homoplasmy. Bidirectional sequencing of purified PCR products confirmed the A to G substitution. The mutation was absent in 386 individuals of the control sample. To our knowledge, this is the fifth family described where this type of NEPPK is caused by the mutation A7445G.

P0016. Cases of rare sex chromosomes' anomalies

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We observed two cases of abnormality of sex chromosomes. Karyotypes were similar. They were mosaic and the first clone was 45,X in both cases, the second clone was 46,Xidic(Y) in one case, and 47,XYY in another case. But phenotypical expression differed in this cases.
First proband was a 12-years old girl. She was consulted because of short stature and retarded puberty. There were no similar cases in the pedigree. Short stature was observed from the early childhood. The common phenotypic features of Turner syndrome were revealed: proportional short stature, palpebral fissures slant up, epicanthic folds, short webbed neck, low posterior hairline, broad thorax, wide-spaced nipples. IQ was normal. Genitalia was formed as female, but mild hypertrophy of clitoris and hyperpigmentation were presented. Sonography showed the absent uterus and streak ovaries highly situated in the pelvis. Elevated gonadotropins were found. Cytogenetic analysis revealed mosaic abnormality of sex chromosomes: 45,X/46,Xidic (Y)(p1.1). The parents' karyotypes were normal.

Another patient was a 1-year old child, had been registered as a male at a birth. The reason of consultation was ambiquous type of genitalia. It was a sporadic case in the pedigree. He was the only child from the pregnancy complicated with toxicosis . His parents were young and healthy. He had urogenital sinus, significant hypertrophy of clitoris, formed as a penis, enlarged labia, surrounded the clitoris. Testes were not palpated. Sonography revealed an uterus. Gonades were not visualized. The child had proportional short stature. No other dismorphological features were revealed. The karyotype was 45,X/47,XYY.

P0017. Exclusion of NOG as the cause of medial talus-calcaneus coalition with short stature: Genetic heterogenity in Tarsal-Carpal Coalition syndrome (TCC).

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Tarsal-carpal coalition syndrome (TCC) is an autosomal dominant disorder characterized by fusion of the carpals, tarsals, and phalanges; short first metacarpals causing brachydactyly; and humeroradial fusion (MIM 186570). The OMIM entry includes medial talus-calcaneus coalition with short stature, reported in a Danish family (Gregersen and Petersen, 1977). We have retraced the family and updated the pedigree. Re-examination of the family confirms the initial clinical data reported, and full penetrance. Mutations in the NOG gene in TCC patients have shown that the condition is allelic to Proximal Symphalangism (MIM 185800) (Dixon et al., 2001), but direct sequencing did not reveal any mutations in this family. The absence of proximal symphalangism or hearing loss in our family prompted us to test markers flanking NOG, which showed that a common haplotype is not shared by affected family members. A genome wide scan is now being performed. We conclude that TCC is genetically heterogenous, and further families should be included in a future mutation screening effort.

Gregersen HN, Petersen GB. Congenital malformation of the feet with low body height: a new syndrome, caused by an autosomal dominant gene. Clin. Genet. 12: 255-262, 1977.

Dixon ME, Armstrong P, Stevens DB, Bamshad M. Identical mutations in NOG can cause either tarsal/carpal coalition syndrome or proximal symphalangism. Genet. Med. 3: 349-353, 2001.

P0018. New cases of autosomal recessive Silver-Russell syndrome

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Silver-Russell syndrome is a heterogeneous malformation syndrome characterised by severe intrauterine and postnatal growth retardation. SRS patients show a small triangular face, relative macrocephaly, asymmetry of head and limbs, clinodactyly V and other less constant features. In 7-10% of patients maternal uniparental disomy (UPD) of chromosome 7 can be determined. In other cases different chromosomal rearrangements have been observed, but no common type of aberration can be identified. However only chromosomes 7 and 17 have been consistently implicated in these cases. The majority of cases of SRS are sporadic, occasionally a familial occurence with autosomal dominant, autosomal recessive or X-linked inheritance is reported.

Here we report on three families with two or three affected individuals indicating an autosomal recessive transmission of the disease. The families were ascertained as part of our ongoing studies on genetic causes of SRS. For these families linkage for the chromosomal regions were carried out for which an involvement in SRS etiology has been hypothesized. While other loci could be excluded, data are compatible with linkage for the microsatellite marker D7S500 in 7q33 for all three families, a region which has been described to be affected by segmental UPD. Further analyses on SRS candidate genes will be focused on this area.

P0019. Mutations in Caveolin-3 gene causing different phenotypes

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Caveolin-3 is the skeletal and cardiac muscle-specific protein of the caveolin gene family and the major component of the caveolae complex. Mutations in the CAV-3 gene cause Limb girdle muscular dystrophy type 1 (LGMD1C), Rippling muscle disease (RMD), Distal Myopathy and Hypercekemia. We report 3 families presenting LGMD1C, 1 family suffering from RMD, 1 family with Distal Myopathy and 2 families with Familial Isolated Hypercekemia. The evaluation of the patients was performed with clinical examination, EMG measurement and serum CK analysis.

Muscular biopsies from the patients were analysed by immunohystochemistry and Western blot with antibodies against dystrophin and dysferlin (Novocastra Laboratories), and caveolin-3 (BD Transduction laboratories, Lexington). Caveolin-3 was reduced in the immunohistochemical and Western blot studies whereas dysferlin was reduced in the immunohistochemical analysis but normal in Western blot.

We sequenced the two exons of the CAV-3 gene using the primers and conditions described by Minetti et al. To date, A G → A transition at nucleotide position 169 in exon 2 in the CAV-3 gene, generating a Val→ Met change at 57 codon (**V57M**) of the aminoacide chain in heterozygous state was detected in one of the families affected by isolated hypercekemia.

The fact of the existence of clinical heterogeneity associated to CAV-3 gene mutations could be due to the specific alterated protein domain. Nevertheless, there are a few mutations described in that gene to conclude about phenotype-genotype correlations.

P0020. Tar syndrom

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Tar syndrome is considered to be an autosomal recessive trait.

The cardinal clinical features are thrombocytopenia (usually with symptoms in the newborn period) and bilateral absence of the radius with the presence of both thumbs.

The thrombocytopenia is congenital and hypomegakaryocytic.Th rombocytopenia gradually improves over the first two years, and platelets may even be in a normal range in adulthood. Viral illness, particularly gastrointestinal viral illness, will often aggravate the thrombocytopenia.

The radius is absent bilaterally in all cases. Anomalies of legs and hips are seen. Congenital cardiac abnormalities are seen in one-third of cases, the most frequent being tetralogy of Fallot and septal defects. Complications are related to thrombocytopenia and symptomatic bleeding. In the past, death occurred before one year of age in 35% to 40% of patients, mostly because of intracranial bleeding. This could be preventable by proper platelet transfusion. Patients seem to have an increased incidence of allergy to cow's milk.

The differential diagnosis includes Holt-oram syndrome, Nager syndrome, pseudothalidomide syndrome and Fanconi anemia. Prenatal diagnosis can be made by ultra sound demonstration of absence of the radius and cordocentesis for platelet counts have resulted in accurate prenatal diagnosis.

Our case was a 4 month infant who died because physician was unable to offer appropriate management. He had absent radius, clubfoot, clubwrist, DDH , and ASD. No check was made of the CBC and the patient died because of intracranial hemorrhage.

P0021. Synostosis of metacarpals IV and V: a diagnostic challenge

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Metacarpal synostosis (MS) IV-V may represent one anomaly among

others in several malformation syndromes, e.g. Apert syndrome, TAR syndrome or FFU complex. On the other hand, non-syndromic cases, uni- or bilateral, not only show genetic heterogeneity (autosomal dominant versus X-linked recessive inheritance) but also marked clinical variability hampering a correct diagnostic classification.
We report a total of 6 patients from 3 families. Clinically, the three index cases show the characteristic MS IV-V with hypoplastic and abducted 5th finger and concomitant cutaneous syndactylies of fingers and toes. Partial duplication of the ring finger in the daughter of typically affected index patient 2 allows further subclassification (Kemp-Ravn type). The index patient 3 presented with bilateral V-shaped MS IV-V while her paternal grandmother and her son demonstrated non-characteristic findings only. This strikingly wide range of manifestations can be a challenge with regard to the diagnostic classification including appropriate genetic counselling and may also explain apparent non-penetrance and „sporadic" occurrence in several families reported.

P0022. Congenital Heart Defects In Down Syndrome: Investigation Of Prevalance And Importance Of Diagnostic Methods In 356 Patients.

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We investigated the prevalence, classification and the diagnostic value of physical examination and echocardiography of congenital heart disease in 356 children with Down syndrome (206 male, 150 female) aged between 1 day-8 years. All patients with Down syndrome followed up in our clinic were included in this prospective study. 30% of the patients were admitted during the newborn period and 48.6% were admitted during 1-6 months period. All patient.s, either with or without a cardiac murmur, were suggested echocardiogram individually. However, only 72% of the patients could be evaluated with an echocardiogram.
The prevalence of congenital heart disease in our patients were 43.5% ; 31.6% of the patients had atrioventricular septal defect (AVSD), 25.1% ventricular septal defect (VSD), and 22.6% atriyal septal defect (ostium secundum). Female infants with Down's syndrome had increased risk for CHD ($p<0.05$). However; there was no statistical relationship between advanced maternal age and the presence of CHD.
Sixteen patients had normal physical examination but an abnormal echocardiogram; 9 patients had ASD secundum, 2 had isolated VSD, 2 had inlet VSD and one had pulmoner stenosis. We noticed that these defects were not serious cardiopathies.
In conclusion; every patient with DS should have cardiac evaluation including the echocardiographical examination, especially during the newborne period. However; in cases when echocardiographical examination can not be performed due to economical and social reasons, the patients may be followed with physical examination findings, electrocardiography and telecardiography beyond the newborn period.

P0023. Molecular-genetic testing in the group of patients suspected of Prader-Willi (PWS) and Angelman (AS) Syndromes.

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Prader-Willi Syndrome is characterized by infantile hypotonia, hypogonadism, psychomotoric retardation and obesity.Angelman Syndrome is disorder with developmental delay, severe speech impairment, gait ataxia and tremulouseness of the limbs and unique behavior. Both disorders are caused by loss of paternally (PWS) or maternally (AS) derived gene expression of chromosome region 15q11-q13.
As the most prevealing cause of these disorders is microdeletion 15q11-q13 (70 %) the fluorescent in situ hybridization (FISH) is powerful tool for its diagnoses. The testing should by completed by molecular analysis of DNA to exclude uniparental disomy (UPD), imprinting mutations (IM) and mutations at UBE3A locus in cases of suspected AS.
In cohort of 44 individuals suspected of PWS and AS, we used DNA methylation analysis to uncover the change in methylation pattern, which is present in cases of deletion, UPD and IM. Out of 34 children with suspected PWS only methylated maternal locus was detected in 10 (30,3 %) children, 9 had microdeletions ~4Mb (FISH). In 1 case the maternal heterodisomy was found and confirmed by STR analysis. Out of 10 AS suspected patients 1 showed inheritance of paternal unmethylated locus only. In this case the paternal isodisomy was prooved by STR analysis.
Three families were provided by prenatal FISH test to exclude microdeletion in the fetus.
We performed molecular examinations aimed at loci of concern in three cases with cytogenetic visible aberration of chromosome 15. Confirmed PWS patients are provided by growth hormone therapy which can alleviate many of the somatic morbidities associated with this condition.

P0024. Clinical variability in a large family with Waardenburg syndrome type 1 carrying a splice site mutation within the PAX3 gene

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Waardenburg syndrome type 1 (WS 1) is an autosomal dominant disorder causing congenital deafness. At present it is considered to be the most frequent cause of congenital sensorineural deafness in humans. Mutations within the PAX3 gene are responsible for the clinical phenotype. Here we represent a four generation family with multiple affected members showing various symptoms of WS who did not suffer from hearing impairment. The phenotype showed a wide intra-familial clinical variability of pigmentary disturbances, facial features and developmental defects. The primary diagnosis was made within the scope of prenatal diagnosis of a spina bifida in an affected fetus. Molecular studies identified a novel splice site mutation within the PAX3 gene in intron 5 in all affected family members. We assume, that this particular splice site mutation IVS5+2 T>C may be not associated with hearing loss in Waardenburg patients.

P0025. Mutations of the TBX5 gene in patients with Holt-Oram syndrome

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Holt-Oram syndrome (OMIM #142900, McKusick 1986) is a rare autosomal dominant inherited disorder involving developmental defects of the heart and upper limbs. Main symptoms are variable cardiac malformations, most of them septation deficiencies and defects of the thumb and radius. The disorder is associated with mutations in the TBX5 gene which plays an important role in the morphogenesis of heart and limbs in vertebrates. To date there are 34 different pathogenic point mutations in the TBX5 gene published but the relationship between genotype and phenotype still remains unclear. We report the results of mutation analysis by direct automated sequencing of the entire coding region of TBX5 including intronic boundaries in 42 patients with phenotype of Holt-Oram syndrome. Phenotype analysis in these patients (13 familial and 29 sporadic cases) demonstrates a high intra- and interfamilial variability. In 11/42 (26%) patients (3 familial and 8 sporadic cases) we could identify a sequence alteration in TBX5, that is possibly predicted to cause the disease. There was no missense-mutation in our cohort. Eight of these mutations are novel mutations, not described before. Five mutations result in a truncated protein product and two are located in intronic splice sites and are most likely of pathogenic importance. Another mutation (3'-UTR +40 A>G), that has been detected in two non-consanginous patients, has an yet unknown effect on the predicted gene product. With our results we can upgrade the number of mutations in TBX5 in HOS patients and further contribute to the understanding the pathogenesis of Holt-Oram syndrome.

P0026. Molecular genetic and clinical survey of Finnish families with dominant optic atrophy (DOA)

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Several linkage studies localise dominant optic atrophy (DOA) to chromosome 3 (3q28) and mutations in the *OPA1* gene encoding a large mitochondrially located dynamin-related GTPase protein have been detected in DOA patients. We have screened all 30 coding *OPA1* exons and exon-intron boundaries from 14 Finnish DOA families by direct sequencing. In ten out of 14 families (71%), eight pathogenic mutations have been identified. Three families share the same mutation as well as a common haplotype surrounding the *OPA1* region suggesting that they most probably have a common ancestor. The other mutations are restricted to one family only, as seen in other populations. Various types of mutations were found – missense, nonsense, and splice site mutations and a small deletion. As there seems to be no clear mutational hotspot in the *OPA1* gene, simple rapid DNA testing can be offered only to those families where the mutation is known. The penetrance of DOA in our family cohort was slightly reduced. No clear genotype-phenotype correlation could be assigned but intra- and inter-familial variability in the clinical outcome was noticeable.

P0027. Further evidence of genetic heterogeneity in Autosomal Dominant Distal Motor Neuronopathy

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Distal hereditary motor neuronopathy (distal HMN) is a genetically and clinically heterogeneous disorder. To date three loci have been identified on chromosomes 7p14, 9q34 and 12q24.We describe a four generation Italian family with distal HMN starting at around 30 years of age with weakness and atrophy of distal leg muscles and pyramidal features. We performed genetic linkage analysis with microsatellites on chromosomes 7p14, 9q34 and 12q24. Negative LOD scores excluded any evidence of linkage to the above-mentioned chromosomes in our family. Moreover, because of pyramidal features in our patients, we performed the linkage analysis to all the known loci for autosomal dominant hereditary spastic paraparesis (ADHSP). The analysis was negative thus excluding that our patients were affected by a complicated form of ADHSP. These data further confirm a genetic heterogeneity within inherited motor neuronopathy disorders.

P0028. Evolving phenotype in pyruvate dehydrogenase complex deficiency

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Pyruvate dehydrogenase complex (PDHc) is an intra-mitochondrial multi-enzyme complex essential for the aerobic oxidation of glucose. The disorder is genetically heterogeneous, however, the majority of patients with a PDHc defect have abnormalities in the major catalytic and regulatory subunit, E1 alpha, which is encoded on the X chromosome. The clinical spectrum of PDHc deficiency is heterogeneous, particularly in heterozygous females and therefore the diagnosis may be difficult. The phenotypic presentation generally includes lactic acidemia, muscle hypotonia, central nervous system involvement, developmental delay, seizures and characteristic dysmorphic features. Patients may have other congenital malformations including corpus callosum hypoplasia or ventricular septal defect. We report on five patients with a PDHc defect, two of which carry a previously undescribed mutation in the gene coding for the E1 alpha subunit of the enzyme complex. The patients, three girls and two boys demonstrated the following unique clinical symptoms consequtively: congenital epilepsy, congenital cataract, colobomas with vermis aplasia, complex cyanotic heart malformation and Bartter syndrome with diabetes mellitus.

P0029. Variation of X inactivation pattern in different tissues in women aged 19-90 years

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The majority of normal females have a random distribution of the X inactivation pattern (XIP). A skewed XIP may arise due to chance, genetic factors or a selection against or in favour of cells with a specific genotype. The frequency of skewed XIP increases with age in blood, but the effect of age on XIP in other tissues or the tissue variation of XIP is not established. We investigated XIP in blood, buccal smear and duodenal biopsies from 92 females aged 19-90 years after informed consent. The correlation between X inactivation and age was 0.200 for blood (p=0.056), 0.243 for buccal smear (p=0.02) and 0.003 for duodenal biopsies (p=0.979). The XIP was significantly correlated between all tissues (0.528 -0.798, p<0.001). The frequency of skewed XIP (≥80:20) was significantly higher in blood (22%) than in buccal smear (7%) and duodenal biopsies (0%) (p<0.001) in the elderly females (> 50 years) but not in the younger females (≤ 50) (frequencies 9%, 3% and 0% respectively). Our results demonstrate a high correlation of XIP between the tissues, a higher frequency of skewed XIP in blood in elderly females and an apparent effect of age on XIP in blood and buccal smear, but not in the duodenal biopsies. Under the assumption that mitotic activity is lower in duodenal biopsies than the other tissues, the results indicate that selection may cause the increase in skewed X inactivation with age.

P0030. Chromosome 3 In The Genetic Clinic

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Between 1/2/81 and 12/31/02 through busy (2002 patient volume 2,797) prenatal and pediatric genetics clinics at the University of South Florida 42,795 probands/families were evaluated. Thirty-five had anomalous chromosome 3. Twenty-three had translocations; 3 each had t(3;5) and t(3;13) respectively; all with different break points. One of them was 47,XXY,t(3;5) mat. Two each had t(1;3), t(2;3),t(3;4),t(3;9),t(3;10),t(3;15), and t(3;17) respectively; and, all with different breakpoints. One with t(3;10) was 47,XX,t(3;10) mat, +18. One each had t(3;6),t(3;8) and t(3;18). Six were maternal, 3- paternal, 6- de novo and 8 had a first degree relative with translocation. Five had complex chromosome rearrangements (CCR): two had 6 breaks each (Clin Genet 42: 135), another had 3 breaks and was the offspring of parents who each had a balanced translocation, mother- t(3;6) and father- t(3;11). (J Med Genet 25: 631). The fourth was de novo with 6 breaks and had t(3;11), inv(3) and t(15;21). (J Med Genet 30: 167). The fifth CCR was with 4 breaks resulting in del(7)(q22q31.3) with t(3;6). The 3 inversions were: 2 maternal and 1 paternal. There were 2 de novo dup(3) (p24.3;p26) and dup(3)(q23;q24), one del (3)(q11q21), and 1 del (3)(q25.3). The latter was maternal and the phenotypes of mother and child were normal. Nineteen were evaluated through the pediatrics clinic and 13 were prenatal. Additional 3 were seen prior to conception for a relative with anomalous chromosome 3. This study demonstrates the wealth of information available in busy genetics clinics having cytogenetics laboratory as part of the genetic program.

P0031. Dysferlinopathies in Spanish families: clinical and molecular studies

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Mutations in dysferlin gene (*DYSF*) cause different muscular dystrophy phenotypes including Limb Girdle Muscular Dystrophy 2B (LGMD2B), Miyoshi Myopathy (MM) and Distal Anterior Compartment myopathy (DAT). These disorders are characterized by autosomal

recessive inheritance, adult onset and elevated levels of serum creatine kinase. *DYSF* gene, that maps to chromosome 2p13, has 55 exons and codifies a protein of about 237 kD that is located in the sarcolemma. The function is unknown but recent studies have demonstrated the involvement of dysferlin in membrane reparation events.
We report the study of 27 families clinically diagnosed as MM, LGMD2B and DAT. Dysferlin expression was studied by immonohistochemistry in muscle biopsies and by Western blot analysis in peripheral blood monocytes CD14+. The absence of dysferlin in monocytes always correlated with absence of the sarcolemmal staining in the muscle biopsy. We did not find reduction of expression either in the monocytes or the muscle biopsy in any of the patients with confirmed dysferlinopathy.
The screening of mutations in *DYSF* gene was performed using the analysis of genomic DNA extracted from total peripheral blood and cDNA was obtained from monocytes. Obtained cDNAs were amplified using primers that covers the 55 exons in 14 fragments and directly sequenced. Study of genomic DNA was done to check mutations found in monocytes RNA.
We identified 12 different mutations: 5 missense, 3 in-frame and 4 stop codon mutations.Three of them were never described elsewhere and in some cases the three different phenotypes are caused by the same mutation.

P0032. Mutation analysis of the CREBBP gene in patients with Rubinstein-Taybi syndrome

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Rubinstein-Taybi syndrome (RTS) is a well-defined autosomal dominant disorder featuring short stature, typical face and skeletal anomalies (broad angulated thumbs and halluces), and mild to moderate mental retardation. Occasional signs include microcephaly and visceral malformations. RTS is caused by mutations of the gene for CREBBP, a nuclear protein that modulates the chromosomal structure by histone modifications and controls the initiation of DNA transcription. Extending a previous report (J Med Genet 2002,39: 496-501), we report here the results of genomic sequencing in 45 unrelated subjects. Clinical diagnoses included RTS (Group A; 30 subjects), possibly RTS (Group B, ≥1 typical sign(s) subtle or absent; 8 subjects), and probably not RTS (Group C, isolated features of RTS; 7 subjects). Causative molecular mutations of CREBBP were found in 16 subjects from Group A (53%) and in 2 individuals from Group B (25%), but not in Group C. The majority of CREBBP mutations were unique and distributed throughout the CREBBP gene, with no real hot spots. Eleven mutations found in Group A were novel, i.e. have not been described previously. In Group B, findings included two de novo substitutions (Y1175C, N1978S) in conserved domains, supporting the existence of incomplete/mild RTS (atypical face in one subject, normal stature and low-normal intelligence in the other). Two other substitutions (L551I, Q2208H), both novel, represented polymorphisms without clinical significance. Although a FISH-based test for RTS is generally available, complexity has hindered the clinical implementation of a molecular-based test.We suggest a tiered approach may be desirable.
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P0033. Assessment of tissue depths over anatomical landmarks on the face

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Recognition of the majority of syndromes requires the ability of the clinician to be able to firstly remember previous patients (or patient descriptions) and to be able to relate these to the patient in question. This process is relatively inefficient and many different techniques have been developed to try and improve this process.
We have used three-dimensional MRI imaging of the face in ten normal subjects as a preliminary investigation into the possible use of this technology to assist in syndrome identification. The initial study compared three-dimensional MRI imaging with three-dimensional photographic imaging (which we had previously shown to be at least as accurate as physical anthropometry). No difference was detected between the three dimensional coordinates derived from photogrammetry and those deduced from MRI.
MRI imaging also permits analysis of other parameters of facial structure. We have analyzed the tissue depth over the anatomical landmarks in the ten subjects. Work on the precision of measurement and inter- and intra-observer variability is in progress. There are some interesting differences between tissue depths previously reported on ultrasound and those observed by us on MRI.
In addition, work is in progress to relate intracerebral structures to external facial structures.

P0034. Increased recurrence risk in congenital disorders of glycosylation (CDG) due to a transmission ratio distortion.

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Congenital Disorders of Glycosylation type Ia (CDG-Ia), the most frequent, recessive inborn error of glycosylation, is caused by mutations in the phosphomannomutase gene PMM2. The high carrier frequency (1/79) of the frequent R141H mutation, is in contrast with the severity of the disorder. We have now evidence that a transmission ratio distortion is at the basis of this high carrier frequency.
In 81 pregnancies among carriers of PMM2 mutations, genotyped in the context of prenatal diagnosis, the percentage of affected foetuses (37%; 30/81 Binomial Distribution p (X>=37%)=0.011) is higher than expected based on Mendel's second law. This means a positive selection of 59.2% (Binomial Distribution p (X>=59.2%)=0.011) for the mutation bearing alleles compared to the normal alleles. Specifically, carriers of R141H also transmit their mutation in 58.8% (Binomial Distribution p(X>=58.8%)=0.09) of the pregnancies.
It suggests that the drive of the mutated alleles relates to a reproductive advantage at the stage of gametogenesis, fertilization or embryogenesis rather than to a resistance to environmental factors during infant or adult life. Such a transmission ratio distortion has not been described for any recessive or metabolic disorder before. Given the importance of glycosylation at all stages of human reproduction, the positive selection might be explained by hypo- or aberrant glycosylation.
The practical implication of this segregation distortion is an increased recurrence risk in CDG-Ia families ranging from 43% in Scandinavian families to 37% in other European families.

P0035. The Genetics of Vesicoureteral Reflux.

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Vesicoureteral reflux (VUR), the reverse flow of urine from the bladder into the ureter and kidney, is a common disorder, found in 1-2% of children. VUR can cause kidney damage and is the most common cause of end-stage renal failure and severe hypertension in children. It is caused by a shortening of the segment of the ureter which runs through the submucosal layer of the bladder wall. The milder grades of reflux commonly resolve with time but more severe grades require medical or surgical intervention. VUR occurs frequently in families, suggesting that it is inherited but the mode of inheritance is unknown.
We have collected 750 DNA samples from over 165 families with more than one child affected with VUR. We are using this resource in an affected-sib-pair approach to search for VUR gene(s). Although no VUR genes have yet been identified, we have excluded all the loci suggested by the genome scan reported by Feather *et al (Am J Hum Genet* **66**:1420-5, 2000) and generated several exciting new leads.

Some of the candidates we have looked at to date are the uroplakins, a family of proteins that physically strengthen the bladder wall. We also looked at the KAL1 gene which causes Kallman syndrome, a condition sometimes associated with VUR. We have recently started a complete genome scan using 4,500 SNP, to identify the loci involved in VUR. This analysis will map VUR susceptibility gene(s), which will a be the first step towards determining the molecular basis of this disorder.

P0036. Prader-Willi syndrome in Taiwan: Diagnosis and Management

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Prader-Willi syndrome (PWS) is a complex disorder affecting multiple systems with many manifestations. We report on our experience in PWS in Taiwan. A three-phase screening protocol has been launched since 1999. Patients with clinical suspicion are referred to Identification Centers for further evaluation, and their samples are sent to the Diagnostic Center. M-PCR is used as 1st-phase screening. If M-PCR was positive, FISH is to be done for detecting deletion. When FISH is negative, microsatellite analysis will be performed to detect UPD. We cannot identify imprinting center mutation. All the cost is fully supported by government subsidiary and a research fund.

From Jan. 1995 to Dec. 2003, forty-seven cases (M:F=22:25, age 2M-21Y) were collected. Birth history, clinical presentation, laboratory and cytogenetics/molecular study results were analyzed. Mean birthweight was 2433gm. Obvious overweight was found in 21/44(48%), and GH deficiency in 20/27(74%). GH therapy is applied in 7/47. Age at diagnosis before age 3M was 16/47(34%), and the rate increased to 67% in recent 5 years. All patients had neonatal hypotonia and hypogonadism. Other symptoms were as follows: characteristic facial features 31/47(66%); rapid weight gain after age 1Y 38(81%); developmental delay 42(89%); hyperphagia 39(83%); behavior problems 33(70%); short stature in 24(51%); hypopigmentation in 42(89%); small hands/feet in 44(94%); skin picking 28(60%) and speech problems 26(55%).

Molecular study revealed deletion in 33/35(94%), one UPD (3%), one probable IC mutation (3%). The progress in early diagnosis helps improve quality of care and better prognosis. GH therapy is still a controversial issue in our country.

P0037. Mutational analysis of the CHRNA4 and CHRNB2 genes in a family with autosomal dominant nocturnal frontal lobe epilepsy

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is included among idiopathic epilepsies. This syndrome often misdiagnosed as nightmares or parasonnies, is characterized by clusters of brief nocturnal motor seizures with a frontal lobe semiology, occurring during non-REM sleep. ADNFLE is clinically homogeneous, even though intra- and interfamilial variability in seizure severity, age of onset (usually in childhood) and specific frontal lobe seizure manifestations can be observed. It is transmitted as an autosomal dominant trait with incomplete penetrance. Three loci, ENFL1, ENFL2, and ENFL3 have been associated with ADNFLE. The locus ENFL1 contains the CHRNA4 gene, three different mutations in the CHRNA4 gene have been described, this gene coding for the alpha subunit of the neuronal nicotinic acetylcoline receptor (nAChR) and located on chromosome 20q13.2. The ENFL2 locus have been mapped by linkage analysis in a single family to chromosome 15q24 region. More recently, a third locus for ADNFLE, ENLF3, has been identified on chromosome 1q21. Two different mutations, affecting the same aminoacid residue, were found in the CHRB2 gene coding for the β 2 subunit of the nAChR.

In this study we report a new 10-member, three-generation family from Calabria with ADNFLE. All index patients were screened for CHRNA4 and CHRNB2 mutations by sequencing. We sequenced the exons and the corresponding exon-intron boundaries of CHRNA4 and CHRNB2 genes in the patients of the family ADNFLE and identified polymorphisms but no mutations. These data, suggested that CHRNA4 and CHRNB2 gene are not involved in the aetiopathogenesis of ADNFLE in this family.

P0038. Fetal Alcohol Syndrome - Clinical study of 21 pacients

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Fetal alcohol syndrome (FAS) is the result of ethanol teratogenesis. Clinical expression is extremely variable, the main features being dysmorphic face, CNS abnormalities, pre and postnatal growth retardation.

We have studied 21 patients (13 females and 8 males) with FAS in order to appreciate the frequency of different clinical features and to present some particularities. The study revealed high incidence of threatening miscarriage (9,09%), prematurity (41,17%) and IUGR (59,09%). Growth deficiency accentuated with age. The frequencies of different clinical signs were: epicanthal fold (18,18%), ocular (18,18%) and auricular (13,6%) defects, microretrognathia (40,9%), cardiac (40,9%), renal (9,09%) and genital (13,6%) anomalies, nipples anomalies (18,18%), hernia (13,6%), sacro-coccygeal sinus (13,6%), hipertrichosis (4,54%), clinodactyly of V finger (22,72%), hypoplastic nails (22,72%), talipes (4,54%). Some of the particular features will be illustrated.

In conclusion, we have found a high incidence of prematurity (especially for girls), a higher frequency of heart defects in girls compared to boys and a higher frequency of sacro-coccygeal sinus in boys.

P0039. Classical Smith-Lemli-Opitz syndrome in a 6 months old girl with only borderline 8-dehydrocholesterol elevation

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Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive malformation syndrome characterized by mental retardation, congenital anomalies, and growth deficiency. The incidence is approximately 1 in 40000 to 1 in 60000. The syndrome is caused by a block in cholesterol biosynthesis at the level of 7-dehydrocholesterol reductase (7-DHCR), which results in elevated levels of the cholesterol precursor 7-dehydrocholesterol (7-DHC) and its isomer 8-dehydrocholesterol (8-DHC). Recently, three patients with a very mild form of SLOS were identified with slight elevation of 7-DHC and 8-DHC (Langius et al. Am J Med Genet 122A, 24-29, 2003).

We report a 6-months old German girl with classical features of SLOS: microcephaly, bilateral postaxial hexadactyly, bilateral cutaneous 2nd and 3rd toe syndactyly. Subsequent biochemical analysis by gas chromatography mass spectrometry (GCMS) revealed normal cholesterol concentration, a 7-DHC level in the upper normal range and a slight elevation of 8-DHC. Cholesterol levels of the parents were normal. We requested molecular analysis. The result revealed compound heterozygosity for the known mutation, W151X, and a novel mutation, I178F, in the 7-DHCR gene. To our knowledge, this is the first case with slightly elevated 8-dehydrocholesterol and verified mutations in classical SLOS. Enzyme measurements in cultured skin fibroblasts are pending.

Our findings give evidence that in a patient with a suspicious phenotype of SLOS, the biochemical analysis of plasma sterols may be equivocal even in the classical phenotype. Therefore, in such cases, mutation analysis or determination of enzyme activity in fibroblasts is indicated. The incidence of SLOS may be higher than predicted.

P0040. Poland Syndrome - Clinical study of 15 patients

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Poland syndrome is a disruption expressed as unilateral absence of sternal head of pectoralis major, ipsilateral limbs defects (usually synbrachydactyly), chest wall defects, absence of nipple/breast and heart defects when the abnormalities are located on the left side; hand defects may occur without absence of pectoralis muscle.
We have studied 15 patients with Poland syndrome in order to appreciate the frequency of different features and their importance for the diagnosis. The group is formed of 12 boys and 3 girls, aged 0-27y. Most of the cases were diagnosed in the first year of life.
Our study showed that males are more frequently affected than females (12:3), right side is more frequently affected (right/ left side : 7/5). The absence of pectoralis muscle was associated with synbrachydactyly (12 cases), short upper limb (5), absent phalanges (1), absent nipples (3), rib abnormalities (1), microcephaly (1), macrocephaly (1), facial asymmetry (2), facial palsy (1 case), auricular anomalies (3), vertebral abnormalities (1), mental retardation (2), epilepsy (1), and nephrotic syndrome (1). Family history was negative in all the cases. Pregnancy was abnormal in 6 cases.
In conclusion, we present a clinical study of 15 patients in order to appreciate the most suggestive and important features for this diagnosis.

P0041. Interstitial deletions in chromosome 7q - two case reports

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Several cases with distal deletion in the long arm of chromosome 7 are described in the literature; most of them include the terminal end of the 7q-arm. The clinical features found in the literature are very variable; frequent findings are short stature, microcephaly, prae- and postnatal growth retardation, mental retardation, cleft lip and/or palate up to holoprosencephaly (Schinzel, 2001). So far there´s no phenotype-genotype correlation for a (7q-) syndrome apparent. Interstitial deletions of 7q35 are rare (Fryns, 1988; Verma, 1992; Fagan, 1994). Here we report on two new cases.
Case1 is a newborn girl who was referred for karyotype analysis with a suspected diagnosis of cri-du-chat-syndrome. Examination at 5 months showed several dysmorphic features (deep infraorbital creases, small nose with anteverted nares, long philtrum, retro-/micrognathia, high palate, short neck; muscular hypertonia).
The karyotype analysis showed an interstitial deletion on one chromosome 7 with the proximal breakpoint in 7q34 and a distal breakpoint in 7q36.
Case 2 is a newborn boy with tetralogy of Fallot and mild dysmorphic facial features (long philtrum, retro-/micrognathia, low-set slightly posteriorly rotated ears, short neck). Standard karyotype analysis and FISH for CATCH22 should be done for exclusion of a del(22)(q11.2).
Our analysis results in the following karyotype:
46,XY,del(7)(q34q36.1).ish7(wcp7x2),10(wcp7-2x)
Examination of his parent´s chromosomes revealed that the 7q35-deletion was due to a balanced insertion [ins(10;7)(q22.1;q34q36.1)] in the proband´s mother.

P0042. A rapid microarray based whole genome analysis for detection of uniparental disomy

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To date, uniparental disomy (UPD) with phenotypic relevance is described for different chromosomes. It is likely that additional not yet identified UPD phenotypes exist. Due to technical difficulties and limitations of time and resources, molecular analyses for UPD using microsatellite markers are performed only in cases with specific phenotypical features and only a few markers are used. Especially screening for segmental UPD requires a larger number of informative markers for a given chromosomal interval. In this study, we made a whole genome UPD search based on a microarray genotyping technique. We analyzed single nucleotide polymorphisms (SNPs) using GeneChip® Mapping 10K Arrays in three unrelated families with children having Prader-Willi syndrome (PWS, maternal UPD15, OMIM 176270), Angelman syndrome (AS, paternal UPD15, OMIM 105830), and Silver-Russell syndrome (SRS, maternal UPD7, OMIM 180860), respectively. SNPs showing opposite homozygosity in the child and in the parent not involved in UPD were indicative for UPD. Our results demonstrated the presence of UPD in these patients with the diagnosis of maternal UPD15, paternal UPD15 and maternal UPD7 as expected. In cases of AS and SRS, reduction to homozygosity of all SNPs along the chromosome was indicative for a complete isodisomy due to a somatic error. In case of PWS, clusters of homozygous and heterozygous SNPs along the chromosome 15 indicated a meiotic error.
We conclude that array-based SNP genotyping is a fast, cost-effective and reliable approach for whole genome UPD screening.

P0043. Multiple circumferential skin creases, facial dysmorphism and mental retardation - a new patient and familial observation

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Congenital circumferential skin creases of the limbs are a rare feature. They are seen as part of the „Michelin tire baby syndrome". Amniotic band sequence can also result in circumferential skin creases.
We report on a girl with skin creases, dysmorphic facial signs and psychomental retardation. The girl was born in the 39th gestational week with a birth weight of 2360 g (<3rd centile), and a length of 45 cm (< 3rd centile). She was the first child of non-consanguineous parents. The skin showed symmetrical semicircumferential creases on the limbs. On physical examination at the age of 1 5/12 years height was 77,5 cm (10th centile), and head circumference 45,5 cm (10th centile). Craniofacial anomalies included blepharophimosis, epicanthal folds, broad nasal tip, puffy cheeks, small posteriorly angulated and low-set ears. Psychomotor development was retarded. Cytogenetic analysis revealed a normal female karyotype 46,XX. Facial appearence of the mother was similar to that of the patient. A maternal aunt had skin creases in infancy by history. The mother, maternal aunt and uncle visited a school for mentally retarded children.
Symptoms in the girl reminiscent of the patient reported by Cohen et al. (Clin Dysmorphol 2:39-46, 1993) and reviewed by Elliott et al. (Am J Med Genet 62:23-25, 1996) and the patient reported by Leonard (Am J Med Genet 112:91-94, 2002). Their male patients had congenital anomalies, psychomotor retardation and circumferential skin creases. Facial features of their patients were similar. Our familial observation suggests the possibility of an autosomal or X-chromosomal dominant inheritance.

P0044. New detection methods identify atypical deletions in Williams-Beuren Syndrome patients

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The incidence of Williams-Beuren syndrome (WBS, OMIM#194050) is estimated to be 1/20,000 and sporadic de novo occurrence is usual. Molecular basis of the syndrome is either a heterozygous microdeletion, or rarely inversion at chromosome band 7q11.23. WBS is a neurodevelopmental disorder characterized by mental retardation with a unique cognitive and personality profile. The cognitive hallmark of WBS individuals is a dissociation between language (relative strength) and visual-spatial cognition (profound impairment). We hypothesize that the absence of one copy of certain but not all of the approximately 25 genes that map within the WBS common deletion region contribute to the neurodevelopmental abnormalities of the complex WBS phenotype. Hence, detailed molecular characterization

of the deletion together with precise cognitive profiling will enable us to investigate the molecular basis of a complex cognitive behavior. The development of alternative methods for faster, higher throughput, accurate and semi-automated diagnosis of cytogenetic abnormalities is highly desirable. We have developed and compared two novel methods for the detection of aneuploidies based on pyrosequencing of paralogous sequences and quantitative real-time amplification of genomic DNA. Both methods have advantages over microsatellite-based detection of aneuploidies, since the tests work on all DNAs, irrespective of informativeness of polymorphisms, and the detection of deletions does not require the parental DNAs. Using these methods we studied a cohort of 162 Italian WBS patients and readily identify three patients with atypical deletions. This molecular analysis, alongside physical examinations and cognitive tests allows to pinpoint, which genes contribute to which WBS deficit.

P0045. Fraser syndrome - the clinical findings in a mildly affected 6-year-old girl.

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Fraser syndrome (FS) is an autosomal recessive (MIM 219000) well delineated multiple malformation complex characterised by cryptophthalmos, cutaneous syndactyly, malformations of the larynx and genitourinary tract, craniofacial dysmorphism, orofacial clefting, mental retardation, musculoskeletal anomalies. McGregor (2003) located the FS gene to 4q21 and identified 5 mutations in the FRAS1 gene.
We present the clinical findings of a 6-year-old girl affected with FS. The patient is the only child of a healthy nonconsanguineous couple, her two elder halfsibs are healthy. The pregnancy was uneventful, birth weight was 3250 g, length 51 cm, OFC 34 cm. First examination of the newborn girl detected rightsided microphthalmia, coloboma of upper eyelid, symblepharon; malformed and mild hypoplastic ears, atresia of the right external auditory canal; syndactyly of hands and feet.
Reexamination at the age of 6 years showed good physical and mental development, microphthalmia, coloboma of upper eyelid and symblepharon of the right eye, malformed ears, atresia of the external auditory canals and moderate conductive hearing loss. There were syndactyly II-IV of hands and II-V of feet. Ultrasonography detected aplasia of the right kidney and mildly enlarged left kidney. The girl had also hypoplasia of the labia majora, but US-examination of uterus and gonads presented normal results.
Our patient definitely fitted three of the major and two of the minor diagnostic criteria of FS, proposed by Thomas, et al.(1986), although all symtoms are mild or moderate.
These findings evidence the clinical variability, associated with Fraser syndrome. Genetic heterogeneity of FS will be discussed.

P0046. Microcephalic osteodysplastic primordial dwarfism (MOPD I/III) in a newborn girl with urogenital anomalies and unusual joint malformations

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Microcephalic and osteodysplasic primordial dwarfism (MOPD) belongs to the genetically heterogeneous and autosomal recessively inherited Seckel syndrome group. According to the literature MOPD I and III were considered to represent the same disorder, share clinical and radiological manifestations, and are clearly different to type II based on radiological and clinical signs.
Here we report on a newborn girl affected by MOPD I/III. The patient is the first child of healthy, consanguineous Turkish parents. Retrospectively, a sister of the mother, who died at the age of 4 months, was diagnosed as also affected by MOPD I/III.
The pregnancy was complicated by severe growth retardation and oligohydramnios. The girl was born at term by cesarean delivery. Birth weight (1060g), length (31,5cm), and OFC (22,3cm) were all far below the 3rd centile. The most striking features were severe microcephaly with sloping forhead, nearly closed anterior fontanelle, prominent occiput, alopecia, prominent eyes, and small ears. The brain showed microlissencephaly and agenesis of corpus callosum. In addition, a small ventricular septal defect, a right-sided multicystic dysplastic kidney, and hypoplastic labia minora were present. Osteodysplastic abnormalities included bowed femora and humeri, dysplastic pelvis with hip dysplasia, and instability of the knees with abnormal movements and dislocation, which has not been reported in MOPD I/III.

P0047. Hypoplastic glomerulocystic kidney disease (GCKD) in a Dutch family

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Glomerulocystic kidney disease (GCKD) is a rare renal disorder which occurs in a sporadic form and a familial form with autosomal dominant transmission. It is characterized by cystic dilatation of the Bowman space and the initial proximal convoluted tubule. GCKD can be associated with either hypoplastic or normal sized kidneys. Glomerular cysts are seen in various renal cystic diseases, such as autosomal dominant polycystic kidney disease (ADPKD), cystic renal dysplasia and the autosomal recessive ARPKD. In diagnosing GCKD exclusion of other cystic renal disorders is therefore important.
There is genetic heterogeneity in familial GCKD. A subgroup of familial hypoplastic GCKD has recently been found to be caused by mutations in the gene encoding hepatocyte nuclear factor-1-beta (*HNF1B*). In addition to GCKD all affected family members suffer from maturity onset diabetes of the young subtype 5 (MODY5).
Here we report on the clinical and pathological features of a father and daughter from a Dutch family who are both heterozygous for the 477delT(P159fsX) mutation in the *HNF1B* gene. Renal biopsies of the father's kidneys show hypoplastic GCKD on histopathological examination. Renal function is moderate to severely impaired. Histologic examination of a nonfunctional removed kidney of the daughter shows a histologically distinct pattern of multicystic renal dysplasia rather than glomerulocystic disease. The remaining kidney, with relatively normal renal function, shows multiple small cortical cysts and echodensity with impaired cortical and medullar differentiation on ultrasound. This family will add to our knowledge of the variable clinical expression of mutations in the *HNFB1*-gene.

P0048. Clinical evaluation of 49 patients with Oculo-Auriculo-Vertebral Spectrum (OAVS) - development of a clinical scoring system and a new classification

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Oculo-Auriculo-Vertebral Spectrum (OMIM164210) is a phenotypically heterogenous disorder characterized by hemifacial microsomia, anomalies of the eye (e.g. epibulbar tumours) and the ear (e.g. microtia), and defects of the vertebral column. Most often, only one side is affected, and in patients with bilateral OAVS, one side is often more severely affected than the other. Most cases are sporadic, but rare familial cases as well as the transgenic *Hfm* mouse model suggest that OAVS may have a genetic basis.
We evaluated the clinical data and photographs of 49 non-related, cytogenetically normal patients with OAVS, all presenting with microtia as a minimal diagnostic criterion. According to the additional signs, the patients were divided into five subgroups:

Subgroup	Clinical findings	Number of patients
1	Microtia	u: 3 b: 9
2	Microtia + mandibular hypoplasia	u: 14 b: 8
3	Microtia + mandibular hypoplasia + vertebral anomalies	u: 2 b: 3
4	Microtia + mandibular hypoplasia + epibulbar tumours	u: 2 b: 4
5	Microtia + mandibular hypoplasia + epibulbar tumours + vertebral anomalies	u: 0 b: 4
		Total: 49

u: unilateral, b: bilateral involvement
Patients of subgroups 3 to 5 more often present with additional malformations, including other anomalies of the eye, congenital heart defects, brain anomalies, microcephaly, short stature, oral clefts, anomalies of extremities and the genito-urinary system. Male to female ratio and ratio of left to right sided mandibular hypoplasia both were 2:1. A scoring system was developed to estimate the severity of the condition.
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P0049. Bloom's Syndrome in a Iranian family

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The patients are two children in a family. The boy was 3-year-old and the girl was 4.5-year-old.Their parents are third degree family relationship. In these cases clinical features include severe growth retardation, small face, big nose, photosensitivity, telangiectatic erythema, high pitched voice and dolichocephaly. Cytological studies revealed chromosomal aberrations and the high frequency in sister chromatid exchanges.
Their mother was pregnant when she referred to us, she had a twine pregnancy but spontaneous abortion occurred at 17 wk gestational age. The incidence of spontaneous abortion increased in affected fetuses.
The patients are also at a high risk for malignancy. Although early diagnosis of leukemia does not elevate the chance for curative treatment, but these children that now are 5.5 and 7 years old are permanently observed for early diagnosis of malignancies included any type of leukemia.

P0050. Mutation analysis of MECP2 and determination of the X-inactivation pattern in Hungarian Rett Syndrome Patients

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Mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) have been associated with the causation of Rett syndrome, an X-linked dominant neurodevelopmental disorder with characteristic, although variable phenotype. Since no consistent correlation is known yet between the type of mutation and the clinical manifestation or outcome, other modulating effects have been investigated to understand genotype-phenotype correlation. It was suggested that the type or domain location of the mutation, and X-inactivation might influence the phenotype through complex interactions. Recently, a higher rate of skewed X-inactivation than expected was reported (Weaving et al, 2003). This finding prompted us to report on our similar observation. We performed mutation analysis of MECP2 in 27 Hungarian Rett syndrome patients and found mutation in 19 cases (70%). Nine previously described mutations were detected in 16 patients (R294X in three patients, R106W, R133C, R168X, T158M and R270X in two patients each, P152R, R255X and 1157del41 once each) and three novel mutations in 3 patients (276insG, 1160del7, 1121del191; 1332del9). Of the 19, 15 patients have the classical and 4 the atypical form of the disease. X-inactivation analysis was performed in 15 patients with mutation. In 3 patients X-inactivation pattern was not informative, in 5 patients random, and in 7 patients non-random X-inactivation was found. The incidence of skewed X-inactivation (7/15) is similar to that reported by Weaving et al. Our results support the suggestion that in Rett syndrome patients with confirmed mutation skewed X-inactivation is a significant modulating factor in about half of the cases.

P0051. Familial perniosis – a newly recognized autosomal dominant genodermatosis

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We describe a large nonconsanguineous German family with 48 members over 5 generations of which 16 are affected with a hitherto unrecognized autosomal dominant genodermatosis. The clinical picture consists of distinct painful purple-red inflammatory lesions in acral locations such as fingers, toes, nose, cheeks, and knees induced by a combination of cold temperature and moisture. The lesions may ulcerate, are associated with arthralgias, and may become hyperpigmentated after healing. The disease onsets in early childhood and tends to improve in late adulthood. Histologically, lesions are characterized by unspecific vasculitic changes with deposits of complement and immunoglobulin and absent hyperkeratosis. Thus, the clinical and histological findings are consistent with either perniosis or Chilblain lupus, a rare cutaneous form of lupus erythematodes. Extensive clinical, histological, and serological investigations of 3 patients could exclude the presence of antinuclear antibodies, complement deficiency, cryoglobulinemia, cold agglutinins, Raynaud´s disease, infections, traumatic injury, keratolytic winter erythema, or Lupus pernio. There are no apparent associated systemic changes or manifestations in internal organs. To the best of our knowledge, this is the first description of perniosis or Chilblain lupus as an autosomal dominant genodermatosis. Identification of the genetic causes underlying this phenotype may shed light onto the pathogenesis of common forms of collagen vascular disease such as vasculitis or lupus erythematodes.

P0052. Asymmetric Crying Face Syndrome with a proximal 22q11.2 deletion - a case report

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A 3 year-old patient was referred to us because of facial palsy in combination with mental retardation especially affecting language development. He had a persistent foramen ovale. Because of the facial signs such as up-slanting palpebral fissures, broad nasal bridge, and broad nasal tip we suspected a microdeletion on chromosome 22q11.2. By fluorescent *in situ* hybridization we detected a proximal 1.5 Mb deletion in this region. This type of deletion accounts for less than 10 % of the 22q11.2-deletions while in more than 90 % the common large deletion spanning 3 Mb is observed.
The phenotype of the patient and the demonstration of the deletion led to the diagnosis of Cayler cardiofacial syndrome which is characterised by congenital cardial defects and an asymmetric crying face due to unilateral absence or hypoplasia of the depressor anguli oris muscle. Thus, the initial diagnosis of facial palsy was revised. In our knowledge this is the first patient with asymmetric crying face syndrome caused by a proximal 1.5 Mb deletion in 22q11.2.

P0053. Genetic aspects of Spermatogenic Impairment-Correlation with Clinical Phenotype

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Microdeletion of the long arm of the Y chromosome, are associated with spermatogenic failure and have been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis.
195 infertile males with idiopathic oligozoospermia and azoospermia were included in this study. Cytogenetic and semen analysis was done in each case. Testicular FNAC was collected whenever possible. Of the 195 cases, 22 were identified as Klinefelter Syndrome (KFS), 14 cases were mosaic KF and 6 cases were variant KF, 4 cases had other cytogenetic abnormalities. In cytogenetically normal cases (n=136) microdeletion analysis was done using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). The STS was considered as absent after 3 amplification failures. Twelve of the 136 cases showed deletion of at least one of the AZF loci. Six cases had AZFc deletion, five cases had AZFa and AZFb deletion and one case showed AZFb deletion alone. Two cases with AZFa and AZFb had SCO Type 1 syndrome and 2 cases of AZFc deletion showed hypospermatogenesis and 1 case showed maturation arrest. Variation in testicular phenotype in cases with AZFc deletion is due to multiple copies of the gene presence of autosomal genes. Cases

with AZFc microdeletion show a progressive decline in semen quality thus these cases are counselled to go in for semen cryoconservation. Deletion on Y chromosome make the Y chromsome more prone to secondary larger deletions resulting in worsening of testicular phenotype.

P0054. The Congenital Myasthenic Syndrome (CMS) mutation RAPSN N88K is an ancient founder mutation

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Mutations in various genes expressed at the neuromuscular junction may cause congenital myasthenic syndromes (CMS). The protein rapsyn is involved in clustering acetylcholine receptors (AChR), and mutations in the RAPSN gene result in AChR deficiency at the motor endplate leading to impaired neuromuscular transmission. Recent reports by ourselves and others identified a specific missense mutation (RAPSN N88K), either homozygously or compound heterozygously, in the vast majority of those patients.
In order to corroborate the hypothesis that N88K might be an ancient founder mutation we collected 21 patients carrying N88K. Patients originated from Germany (n=5), Austria (n=1), Italy (n=1), France (n=2), UK (n=10), and India (n=2). Analyzing 21 single nucleotide polymorphisms (SNPs) neighboring RAPSN on chromosome 11p11 revealed a common haplotype for N88K encompassing a distance of about 360 kb. Our findings provide strong support for the supposition that the occurrence of N88K in different European populations is derived from of an ancient founder event.
The frequent occurrence of RAPSN N88K will greatly facilitate the diagnostic process in European CMS patients.

P0055. Proportionate shortness of upper limbs and macrocephaly in Léri-Weill syndrome.

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The major clinical findings in Léri-Weill syndrome are short stature with mesomelia of upper and lower limbs, bowing of radius, Madelung deformity, and muscular hypertrophy. The syndrome is caused by deletions or mutations of the *SHOX* gene located on the pseudoautosomal region of the X and Y chromosome. The girl we describe here was born at term with birth weight 2800 g and length 49 cm. At age 5 month disproportionate shortness was reported to become significant. At age 14 years, her length was 149 cm (3rd-10th centile) with sitting height 82 cm (50th centile), weight was 56 kg (75th-90th centile), and OFC was 56,5 cm (>97th centile). The general aspect with disproportionate shortness, palpable bowing of radius, wrist deformity, and athletic habitus was suggestive for the diagnosis of Léri-Weill syndrome. The diagnosis was confirmed be molecular studies; a complete heterozygous deletion of the *SHOX* gene was detected. In contrast to the common opinion that both, upper and lower limbs are mesomelic short in Léri-Weill syndrome, anthropometric measurements showed that in our patient this was evident for the legs only. The upper legs length was -2 SD and the lower legs length was -3.2 SD. The arms, however, showed proportionate shortness with upper and forearm length of -4.6 SD both. Another finding was the presence of macrocephaly which as far as we know was not described before. These unusual findings in our patient underline the highly variable phenotype in Léri-Weill syndrome and may extend the clinical spectrum of *SHOX*-related disorders.

P0056. Carey-Fineman-Ziter syndrome: a report of a new patient and phenotype delineation

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Carey-Fineman-Ziter syndrome is a very rare disorder characterized by a dysmorphic face, Moebius and Robin sequences, congenital myopathy, and growth retardation. Skeletal anomalies and developmental delay are occasionally present. Only 6 patients have been described so far, two sporadic cases and two pairs of siblings suggesting autosomal recessive inheritance. We report a 9-year old girl with remarkably similar anomalies. Her unusual facial features included high forehead, prominent metopic suture, antimongoloid eye slanting, strabism, high myopia, low-set, poorly shaped ears, hypoplastic alae nasi, short philtrum, cupid shaped, short upper lip, small mouth with receding angles, and Robin sequence. She also had Moebius sequence resulting in distinct functional abnormalities involving eye movement, facial expression, mouth closure, speech, and swallowing. During early infancy difficulties in sucking, failure to thrive and marked hypotonia were noted. She had bilateral talipes equinovarus with hyperlaxity of other joints. Neurological evaluation showed multiple lesions of the cranial nerves, predominantly n. abducens, and bilateral facial nerve palsy, striking atrophy of the lower leg muscles, reduced deep-tendon reflexes and signs of lower motor neuron lesion on EMG which have not been observed in reported patients so far. Her mental development was within the normal range, although motor deficit, slow speech development, short attention span, hyperactivity and disturbances of visual and motor organization and integration were noted. Our patient delineates the features of Carey-Fineman-Ziter syndrome confirming the Moebius-Robin sequence association to congenital neuromuscular disorders.

P0057. Elicited repetitive daily blindness: a new familial disorder related to migraine and epilepsy

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We report a family affected by a new syndrome combining a previously undescribed form of transient total visual loss, “elicited repetitive daily blindness” (ERDB), with familial hemiplegic migraine and epilepsy, apparently segregating as an autosomal dominant condition.
The ERDB phenotype is characterized by multiple daily episodes of transient total visual loss, which are most commonly bilateral. The episodes can be reproducibly triggered by changes in light intensity or in ocular pressure (rubbing, orthostasis) and last 3 to 10 seconds. They are consistently followed by a 30-second period during which amaurosis cannot be reprovoked. The phenotype has an onset early in life and a benign course over many decades, essentially excluding a vascular origin.
In this family, ERDB cosegregates with clinically typical familial hemiplegic migraine; furthermore, several affected individuals suffered from epilepsy in childhood before developing FHM. We propose that that ERDB, FHM and childhood epilepsy represent different manifestations of the same underlying disorder, caused by a single, autosomal dominant defect. It is at present impossible to explain this remarkable phenotype, which is perhaps suggestive of a calcium channelopathy; linkage to the CACNA1A gene, the first gene associated with FHM, was excluded.

P0058. Fetal valproate syndrome in monozygotic twins.

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A pair of monozygotic twins was exposed to sodium valproate during the pregnancy with a daily dose of 1300 mg (500 mg + 800 mg). Repeated measurements of sodium valproate levels of the mother were within the therapeutic range. No folic acid supplementation was used. The twins were born at 38+5 weeks of gestation. The birth weight of twin A was 2930 g (22 %), length 49.5 cm (35 %) and head circumference 32 cm (15 %). Apgar score was 9. He had muscular VSD, undescended right testis, postaxial polysyndactyly of right foot, and obstructed lacrimal ducts. The birth weight of twin B was 2585 g (16 %), length 48.5 cm (23 %) and head circumference 32 cm (15 %). Apgar score was 6/8. After birth he had feeding problems and a nasogastric tube was needed. His heart defect was much more severe with a double outlet right ventricle (DORV), pulmonal stenosis

(PS) and large VSD. In addition, he had postaxial polysyndactyly of feet, which was bilateral, and undescended left testis. At 2 years of age his motor skills were delayed but speech development was normal. Both boys have distinctive facial features of fetal valproate syndrome. Their monozygosity was confirmed by a DNA test. Previously fetal valproate syndrome has been reported in dizygotic twins. To our knowledge, this is the first report of monozygotic twins with fetal valproate syndrome.

P0059. MASA - Is it Time for a New Acronym?

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MASA is an easy to remember and therefore clinically useful term for X-linked hydrocephalus (OMIM 303350) and summarizes the features Mental retardation, Aphasia, Shuffling gait, and Adducted thumbs. It was coined in 1974 by Bianchine and Lewis, long before the diagnosis could be delineated by molecular studies (1992) which showed that mutations in the L1CAM gene cause this special kind of hydrocephalus.
Although case reports suggested a wide phenotypic variability no study so far has given a survey of the clinical course with respect to this distinct form of cerebral malformation.
We managed to retrieve follow-up data from cases who had been reported to or seen in our institution, either because relatives had sought for genetic counselling or via support groups.
Data of 13 boys were analysed:
All affected individuals have adducted thumbs and talipes, two have additional malformations (cleft palate, clinodactyly).
Three individuals did not survive beyond the neonatal period, all remaining cases received a ventricular shunt.
4/10 surviving are able to walk.
6/10 can eat on their own.
6/10 are able to talk.
A rough genotype/phenotype correlation can be derived from the position of the mutation.
In our opinion the term MASA needs to be modified as it suggests severe disabilities and may lead to an incorrect prognosis for affected fetuses and infants. Two thirds of boys with x-linked hydrocephalus are not affected by aphasia but another two thirds are unable to walk. For future use we propose the acronym BATH: Boys with Adducted Thumbs and Hydrocephalus.

P0060. Novel mutation affecting SRY-DNA binding activity associated with 46,XY pure gonadal dysgenesis

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The SRY gene (sex determining region of the Y chromosome) initiates the process of male sex differentiation in mammalians. In humans mutations in the SRY gene have been reported to account for 10-15% of the XY sex reversal cases.
We describe one novel indel mutation in the SRY gene at the patient with pure gonadal dysgenesis (Swyer syndrome). Karyotype analysis was performed on amniotic cells obtained via amniocentesis due to maternal age (37 years). It revealed a 46, XY complement without mosaicism. Soon after birth we observed a discordance between female phenotype and male karyotype. We performed molecular analysis of the SRY gene and we found indel mutation: C146-153del8ins24 which causes G49fsX10. The protein is shorter and has no HMG domain. MRI investigation of lesser pelvis showed no differentiated gonads at the age of 3 months.
Conclusion: In the best of our knowledge this is the fist case of assesing the patient with pure gonadal dysgenesis soonly after birth because of discordance of prenatal male karyotype and female phenotype.

P0061. Study of the WFS1 gene and mitochondrial DNA in Spanish Wolfram syndrome families

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Wolfram syndrome (WS) is an autosomal recessive neurodegenerative disorder characterised by early onset diabetes mellitus and progressive optic atrophy. Patients with WS frequently develop deafness, diabetes insipidus, renal tract abnormalities and diverse psychiatric illnesses, among others. A gene responsible for WS was identified on 4p16.1 (*WFS1*). It encodes a putative 890 amino acid transmembrane protein present in a wide spectrum of tissues. A new locus for WS has been located on 4q22-24, providing evidence for the genetic heterogeneity of this syndrome.
Six Spanish families with a total of seven WS patients were screened for mutations in the *WFS1* coding region by direct sequencing.
We found three previously undescribed mutations c.873C>A, c.1949_1950delAT and c.2206G>C, as well as the duplication c.409_424dup16, formerly published as 425ins16.
Several groups had detected deletions in the mitochondrial DNA (mtDNA) of WS patients. For this reason, we also studied the presence of mtDNA rearrangements as well as LHON, MELAS and A1555G point mutations in the WS families. No mtDNA abnormalities were detected.

P0062. The adult phenotype in Costello syndrome.

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Over 100 cases of Costello syndrome have been reported but only ten cases published of individuals sixteen years and older, so little is known about the natural history of the condition in adult life. We report 12 adults with Costello syndrome ranging in age from 16 to 40 years. In adult life, health problems included benign tumours: multiple ductal papillomata in two women and a fourth ventricle mass in one man, thought to be a choroid plexus papilloma. One patient in this series has previously been published with a bladder carcinoma. There were no other malignancies. Endocrine problems included delayed puberty and osteoporosis. Other health problems were symptomatic Chiari malformations in two patients and adult-onset gastro-oesophageal reflux in three patients. Nine adults had mild to moderate intellectual disability, two individuals had severe intellectual disability and one individual had profound intellectual disability. Nine of the twelve individuals attained some reading and writing skills and there was no loss of skills. This data contributes to the knowledge of the adult phenotype of Costello syndrome and informs the counselling of families.

P0063. Mutations and polymorphisms in the TCOF1 gene in patients with Treacher Collins syndrome

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Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial disorder characterised by malar hypoplasia, micrognathia, macrostomia, auricular defects, conductive deafness and cleft palate. The estimated incidence is 1/50000 live births, with 60% of the cases resulting from *de novo* mutations. The *TCOF1* encodes a protein, 1411 amino acids in length, named treacle. At least 106 mutations spread throughout the *TCOF1* were detected, mostly resulting in a truncated protein product of the gene.

The structure of the *TCOF1* was investigated in 50 patients with TCS and their 36 relatives. The DNA fragments were amplified by PCR and were subsequently subjected to multitemperature single-stranded conformation polymorphism (MSSCP) analysis, followed by real-time PCR, and direct sequencing.
In one patient, sequence analysis of the amplified exon 15 revealed a novel c.2373-2374delAG. This mutation generated a stop codon at aa 794. In another patient, sequence analysis of exon 7 showed a c.786-787delAG. This mutation generated a stop codon at aa 270. Amplification of these fragments using *LightCycler* system revealed different melting temperature between normal and mutated alleles. In exon 15, was identified a novel c.2344C>T transition, resulting in Gln782Ter and in exon 23, a novel c.3880G>T transversion, resulting in a Glu1294Ter. Two polymorphisms were demonstrated in the *TCOF1*: c. 2429 T>C, resulting in Ala810Val in exon 16 and c.3938 C>T in exon 23 resulting in Ala1313Val.
Our results indicate the importance of molecular diagnostics in Treacher Collins syndrome for prenatal and postnatal screening and for genetic counseling.

P0064. A new phenotype with hypomelanosis Ito due to mutations in the *p63* gene?

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Mutations in the human *p63* gene are known to cause either isolated split hand/foot malformation or different syndromes such as EEC-, AEC-, ADULT-, Rapp-Hodgkin- and Limb-mammary syndrome. Recently, we found a missense mutation in the DNA-binding domain of *p63* in a boy presenting ectrodactyly, cleft lip/palate and hypomelanosis of Ito. Hypopigmented skin patches and streaks were located on the trunk, arms and legs. No additional skin abnormality or ectodermal dysplasia was observed. Even though hypomelanosis of Ito is a non-specific phenotype and often caused by chromosome abnormalities, we hypothesize a correlation between hypopigmentation and the *p63* mutation in this case. Our hypothesis is supported by previous observations of individuals with both ectrodactyly and hypomelanosis (Stewart et al. 1979; Riccardi et al. 1980). However, it remains unclear if there is a chromosome or mutational mosaicism in these patients. Interestingly, patients with *p63* mutation-induced ADULT syndrom also have extensive freckling or characteristic hyperpigmentated skin patches. These observations of hypo- or hyperpigmental skin changes in *p63*-associated disorders raise the possibility of a specific *p63* function in the skin and melanocytes.

P0065. Rare case in hemophilia A in a female patient with a 46,X,idic(Xq) karyotype

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Hemophilia A is an X linked, recessive, bleeding disorder caused by a deficiency in the activity of coagulation factor VIII. The disorder is caused by mutations in the factor VIII gene which maps to Xq28.
A 32 year old female patient with 30 kg (‹3 c) weight and 120 cm (‹3 pc) height was referred for diagnostic examination because of a prolonged bleeding observed by casual injuries. Hemophilia A with 23 % VIII factor activity has been diagnosed. Her two male cousins are severely affected by hemophilia A.
The patient was already examined by us before 19 years because of Turner phenotype. The cytogenetic analysis showed 45,X/46,X,idic(Xq)/47,X,idic(Xq),idic(Xq) (10%/84%/6%). At that time no mental retardation or bleeding disorder was present.
The patient was mentally severely retarded; otherwise her phenotype was the same as 19 years before. The ultrasound examination of the pelvic region demonstrated no ovarian and uterus-like structures. At the cytogenetic analysis at 550 band resolution 45,X/46,X,idic(Xq)/47,X,idic(Xq),idic(Xq) (8%/87%/5%) karyotype was proved.
The correlation of genotypic and phenotypic findings at the examinations performed at two different times is striking. Namely, at the age of 13 mental development was at average and no bleeding disorder was established, while at present severe mental retardation and mild hemophilia A were verified.
Considering the change in clinical manifestation together with unchanged ratio of mosaic cell lines is it not unrealistic to assume that X inactivation might play some role in the phenotypic deterioration.

P0066. Phenotypic variability of 22q11 deletion within families

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22q11 deletion is the most common microdeletion. Estimated occurrence is in 1 in 4, 000 live births. The majority of deletions are de novo. 10-28% results from autosomal dominant transmission. In our group of 28 patients the inherited deletion occurred in 9 of them (32%). Maternal transmission has been confirmed in all of 9 cases. In 2 cases there was a prenatal detection of conotruncal cardiac disease; 22q11 deletion was confirmed by FISH analysis after the birth. In one family four members with 22q11 deletion were detected; in other case the patients were monozygotic twins.
Parents and their children show extensive phenotype variability in concordance with other published data. Cardiac defect has been confirmed in all of 9 probands but only in 3 of their mothers, other 2 were observed for heart murmur in their childhood. Facial stigmatization and other symptoms were also highly variable within the families. All of the mothers with 22q11 deletion were mentally subnormal and showed similar pattern of behavior.
Our study confirms the need of examination of parents when the 22q11 deletion is confirmed in their child. The same size of deletion proved by molecular cytogenetic analysis has been documented for the similar cases but cause of such variability of phenotype expression was not yet explained. As a possible explanation of phenotype variability other modifying genetic loci or environmental factors are suggested.

P0067. A case ofschizencephaly with severe mental retardation, cardiac and facial anomalies: a new syndrome?

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Schizencephaly is an uncommon brain malformation characterised by a gray-matter lined cleft extending through the entire hemisphere. Most cases are sporadic but familial occurrence has been described. Schizencephaly is thought to represent a defect in neural migration. Concomitant brain malformations (e.g. polymicrogyria, septum pellucidum aplasia) are common, but the combination with other organ involvement is rare (e.g. septo-optic dysplasia).
We report on a seven year old boy with syndromal disease including unilateral fused lip schizencephaly with epilepsy and hemiparesis, severe mental retardation, peculiar face, pulmonal atresia with right ventricle hypoplasia, and severe feeding difficulties. His three year old brother shows no schizencephaly or other organ involvement but similar facial features and severe mental retardation. Chromosome analyses and subtelomere deletion screening in both children were normal as was selective screening for inborn errors of metabolism in the younger boy. To our knowledge this is the first description of schizencephaly multiple malformation syndrome. The familial recurrence in brothers is compatible with autosomal-recessive or X-chromosomal inheritance, the different expression is suggestive of endogenic and/or exogenic modifiers.

P0068. Early hematopoietic zinc finger gene (EHZF) is disrupted in a boy with multiple congenital anomalies, autism and mental retardation.

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A 3-year-old boy with multiple congenital anomalies, mental retardation and autistic behaviour was previously reported to carry a de novo reciprocal translocation: 46, XY, rcp (2;18)(p13;q11.2). He had intrauterine growth retardation, microcephaly at birth and low birthweight. An history of frequent upper respiratory infections

is associated with a reversed CD4/CD8 ratio. The phenotype is now made up of microcephaly, narrow forehead with frontal bossing, hypertelorism, epicanthic folds, long ears, flat philtrum, cupid bow mouth, narrow palate, single palmar crease on the left hand and 5th-finger clinodactyly.
Using BAC clones, we mapped the translocation breakpoints using fluorescence in situ hybridisation (FISH). Two overlapping genomic clones crossed the breakpoint on the der(18) chromosome, locating the breakpoint region between RP11-958F21 and RP11-467C13. A combined approach with long range PCR and Southern blot analysis allowed us to determine that the early hematopoietic zinc finger (EHZF) gene on chromosome 18 was disrupted by the rearrangement. A single BAC clone, RP11-798J16, crossed the breakpoint on the der (2). Extensive Database research did not show any other gene that might be disrupted on chromosome 2. We suppose therefore that the disruption of the EHZF gene is probably the molecular cause of the phenotype observed in our patient. Nevertheless, we are now evaluating the possibility that the expression of another candidate gene in the vicinity of the breakpoint could be altered by the rearrangement through a positional effect.

P0069. Periventricular nodular heterotopias and dilatation of the ascending aorta in two distinct families: a new condition ?

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We report on five patients from two different families presenting with the association of periventricular nodular heterotopias (PNH) and dilatation of the ascending aorta.
In the first family, our patient was 31 when she was first referred to the genetic clinic. She presented with the association of aortic incompetence and non-epileptic paroxystic neurological manifestations secondary to PNH and arachnoid cyst of the posterior fossa. Her father presented with supra-valvular aortic dilatation repaired at the age of 41, and his brain MRI revealed asymptomatic PNH.
In the second family, our patient went under surgery for dilatation of the aorta at the age of 38. She presented with complex seizures due to PNH associated with mega cisterna magna. Her mother was operated at the age of 54 from a dissection of the aorta, and her daughter presented at the age of 10 with aortic dilatation treated with beta-blocker and PNH.
There were neither clinical features of Marfan nor Ehlers-Danlos syndromes (EDS) in these patients, although distal hypermobility was noted as well as generalised thin skin. Reviewing the literature, we could not identify any similar condition, but the association of dilatation of the aorta and PNH had been previously reported in two patients with Ehlers-Danlos syndrome (Cupo, 1981; Thomas, 1996). Two other patients with EDS had also been described with bilateral focal polymicrogyria (Echaniz-Laguna, 2000).
We think that this association is probably a new autosomal dominant condition, and that there is probably a link between connective tissues anomalies and defect of neuronal migration.

P0070. Black locks-Albinism-Deafness syndrome is allelic to Waardenburg syndrome type 4 and can be ascribed to heterozygous SOX10 mutation

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Waardenburg syndrome type 4 (WS4) is a rare disorder characterised by sensorineural hearing loss, pigmentation anomalies and Hirschprung disease. SOX10 mutations have been reported in this condition when inherited as autosomal dominant trait. Black locks-Albinism-Deafness syndrome (BADS) is a rare autosomal recessive condition, in which EDNRB mutations have been described.
We report on a patient presenting with overlapping features of these two conditions.
She presented with severe hypotonia and constipation from birth, and at 3 months of age pigmented patches appeared over generalised skin and hair hypopigmentation, except for a brown lock. Sensorineural hearing loss was diagnosed at one year. When first referred to the genetic clinic at the age of 4 ½, growth parameters were normal. She was hypotonic and hypermobile, and her gait was ataxic. Neither dystopia canthorum nor iris heterochromia were noted, but there was moderate retinal depigmentation. Sweat, saliva and tears were normally produced. Cardiovascular investigations were normal, as well as anorectal manometry. Brain MRI showed hypersignals in the periventricular region. Mutation screening of SOX10 revealed a de novo heterozygous mutation (P175A).
Our case further expand the clinical variability associated with SOX10 mutations, since it combines features of BADS with neurological signs of WS4 but without Hirschsprung disease. We therefore confirm that BADS is allelic to WS4, and can also be ascribed to heterozygous SOX10 mutations.

P0071. Brachydactyly-short stature-hypertension syndrome: a new case associated with renal and cerebellar vascular malformations

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Brachydactyly-short stature-hypertension syndrome is an autosomal dominant syndrome, was firstly described by Bilginturan *et al*. in 1973. The responsible gene of this syndrome was mapped to 12p12.2-p11.2 (Schuster *et al*., 1996). We report a new case, and demonstrate his cerebellar and renal vascular malformations by magnetic resonance angiography (MRA).
A 16-year-old male was referred to us because of arterial hypertension of unknown etiology. He had attacks of headaches, and epistaxis. Family history revealed that his father had chronic renal failure for 15 years. The father had history of hypertension attacks, but cause of chronic renal failure was unknown. Physical examination of the patient showed short stature, brachydactyly of the hands, and macrocrania with normal mental-motor development. His blood pressure was 150/90 mmHg. Routine blood, urine, renal function and hematologic tests were in normal limits. Echocardiography, abdominal ultrasonoghraphy, and cranial MRI disclosed no pathological findings. X-ray of the hands showed brachyphalangy, cone shaped epiphysis, and especially shortness of the 4th and 5th metacarpals.
Since, previous reports noted that anomalies of intracranial, mesenteric and renal arteries (Chithayat *et al*., 1997 and Litwin *et al*., 2003), we examined the patient by contrast-enhanced MRA. This study showed right aberrant posterior inferior cerebellar artery, and that left renal artery had early bifurcation, irregularity and stenosis of its inferior dominant branch.
Previous reports and our observations indicated that vascular anomalies or possible diffuse vascular pathology are major component of this syndrome, and the responsible genes may be related to generalized connective tissue disorders.

P0072. Two cases of PHACES *(Posterior fossa anomalies, Hemangiomas, Arterial anomalies, Cardiac/aortic malformations, Eye anomalies, Sternal cleft/Supraumblical raphé)* syndrome from Turkey

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Facial hemangiomas, sternal nonunion, supraumblical raphé, vascular anomalies and Dandy-Walker malformation syndrome was described as a new entity by Gorlin in 1994. Frieden *et al*. (1996) suggested use of acronym “PHACE” syndrome, because these cases had **P**osterior fossa anomalies, **H**emangiomas, **A**rterial anomalies, **C**ardiac/aortic malformations, and **E**ye anomalies. When ventral developmental defects such as **S**ternal cleft or **S**upraumblical raphé are observed, the condition is expanded as “PHACES” syndrome. Grosso *et al*. (2004) suggested that the facial hemangiomas may be associated with disorders of the cortical development.
We will present two new cases of PHACES syndrome from different families. First case was a 3-month-old female who had widespread facial, lower lip, tongue and soft palate hemangiomas. She was second child of non-consanguineous parents. The patient was

followed by us, and treated with corticosteroids. By the time she got 4 years old, the facial hemangiomas had been regressed. The second case was a 3-month-old male born to consanguineous parents. The patient had macrocephaly, mental-motor retardation, coarse facial features, macroglossia, and sternal and frontal hemangiomas. Umblical hernia and bilateral hydrocele were also noted. Cranial MRI showed cortical atrophy. Echocardiography were normal in both of our cases. This syndrome may have a broad phenotypic spectrum. Further molecular studies will help us to understand the phenotypic variability. Reported cases indicate a highly significant association between ipsilateral hemangiomas and cerebrovascular, and aortic arch anomalies (Bronzetti *et al*., 2004).
Finally, every case with superficial hemangiomas should be checked for the presence of cardiovascular, intracranial, and eye anomalies.

P0073. Modified allelic replication in genomes of patients with Beckwith Wiedemann Syndrome

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A loss of synchrony in allelic replication timing has been detected in lymphocytes derived from patients with various types of malignancies or premalignancies. As Beckwith Wiedemann Syndrome (BWS) is an overgrowth condition associated with an increased risk to develop malignancies, we used the fluorescence in situ hybridization (FISH) replication assay and evaluated the level of replication synchrony of three cancer-implicated genes (*RB1*, *AML1,* and *CMYC*) in lymphocytes derived from patients with BWS without malignancy. Ten controls and 6 patients with BWS including 2 sporadic individuals - one with paternal uniparental disomy (pat UPD), one with mosaic pat UPD and 4 individuals from the same family with enhancement of the paternal band at H19 locus, were evaluated. In cells derived from controls, each pair of alleles replicated synchronously; yet these same alleles replicated in a significantly increased asynchrony in cells derived from BWS patients. Each gene, which normally displayed synchrony in allelic replication, in the patients' cells displayed loss of synchrony. The loss of replication synchrony, of each gene, in the patients' cells was achieved by an advanced replication of a single allele, which replicated remarkably earlier than its normal scheduled timing. In addition, the second allele showed a significantly earlier replication timing than that normal for the gene. Thus, it is assumed that the BWS condition is associated with activation of cancer-implicated genes that may be the cause for over growth and an increased risk of patients to develop malignancies.

P0074. Beckwith - Wiedemann syndrom: Orofacial and other findings - overview of 15 families

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Syndrome is characterized major features: exomphalos, macroglossia and overgrowth. The locus has been assigned to 11p15.5. Small number of BWSs have abnormalities involving in WT 1 locus in chromosome 11p13. Alterations of region 11p15 have been detected in neoplastic disease and in cancer predisposing syndromes, too. Most of our cases are sporadic. Somatic mosaicism is connected with hemihypertrophy and with paternal isodisomy. We conducted stomatological, genetic and paediatric study to clarify major and minor diagnostic characteristic and longterm expectation. We noted facultatively signs: wall defects and or macroglossia. Many of these infants are born prematurely. We detected no cytogenetic variations, no tumours have been detected. Some candidate tumor supressor genes are relative to Beckwith - Wiedemann syndrome. Long term ultrasound monitoring was provided. Treatment of the craniofacial complications of BWS depend from variable degree of macroglossia. Partial glossectomy has resulted in decreased anterior open bite and mandibular prognathism. The most frequent complications were macroglossia, abdominal wall defect and prenatal and/or postnatal overgrowth. Relative common features are horizontal earlobe creaseas and pits, facial naevus flamneus, nefromegaly with renal medullary dysplasia and hypoglykemia. Hypoglycaemia is a frequent finding during the neonatal period.Relative rare complications are hemihypertrophy, moderate or severe developmental delay and congenital heart defects and polycythemia. The mode of inheritance is complex, but recetly compiled data suggest that it is an autosomal dominant trait of varying expression. Incomplete penetrance may lead to familial BWS being underdiagnosed. Part of apparently sporadic cases had a relative with possible BWS.

P0075. Late and very late-onset Friedreich ataxia in Czech republic

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Friedreich ataxia (FA) is the most common autosomal recessive ataxia, accounting for approximately 50% of all cases of hereditary ataxia. Cardinal features include progressive limb and gait ataxia, dysarthria, loss of joint position and vibration senses, absent tendon reflexes in the legs, and extensor plantar responses.
Onset of FA is before age of 20 - 25 years. In approximately 15% of patients with FA, the age of onset may be greater than 25 years. The age of onset in late onset Friedreich ataxia (LOFA) is between 26-39 years and in very late onset FA (VLOFA) is >40 years.
We examined a series of 490 patients from unrelated families attending genetic testing because of idiopathic, progressive ataxia and inheritance compatible with autosomal recessive or sporadic disease. All patients were of Czech origin except 2 (Slovak origin). 28 patients from the examined group (5,7%) were homozygous for a GAA triplet-repeat expansion in intron 1 of the FRDA gene. The age of onset has been observed between 4-20 years in 18 patients (64%), between 20-25 in 6 patients (21%), between 25-39 years in 3 patients (11,5%), and after 40 years in 1 patient (3,5%).
We demonstrate clinical picture and results of DNA test in 4 detected cases with late and very late onset Friedreich ataxia.
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P0076. A novel autosomal recessive progressive hyperpigmentation syndrome

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We present a family of Iraqui origin with three siblings affected by a novel type of progressive hyperpigmentation syndrome. The generalized hyperpigmentation started in early infancy and increased during childhood. It also affected palms and soles, and the face but spared cheeks. Additional features were dry, itchy and sunlight sensitive skin, dystrophy of toe nails, hair loss, myopia. The remaining family history was unremarkable. Chromosomal analysis at a 500 banding level in one girl showed a normal female karyotype. A similar entity was described as "Melanosis diffusa congenita" with unknown origin by Braun-Falco et al. in 1980. Familial occurrence in that entity suggested autosomal dominant inheritance with reduced penetrance. However, in our family the two affected girls and one affected boy were offspring of a first cousin marriage suggesting autosomal recessive inheritance.

P0077. FISH for detection of 22q11.2 deletion among patients referred for Di George/VeloCardioFacial Syndrome (DGS/VCFS)

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DGS and /VCFS result in the majority of cases from deletion or rearrangement within chromosome band 22q11.2 and there is a wide variability of clinical spectrum. Cytogenetic and FISH analysis was performed in 139 patients (82 males /37 females) aged 10 days-15 years, for the detection of a deletion in the critical DGS/VCFS region. Detailed clinical data were available in 56 cases referred by pediatricians (32/56), cardiologists (20/56) and neurologists (4/56) for one or more of the following: congenital heart defects (47/56), facial

dysmorphic features (37/56), mental retardation/behavioral problems (23/56), multiple congenital anomalies (15/56) skeletal abnormalities (15/56), Hypocalcaemia (10/56), immunological problems (10/56), growth retardation (8/56), hypotonia/seizures (7/56), velopharyngeal insufficiency (5/56) and cleft palate (4/56).Cytogenetic analysis revealed an abnormal karyotype in 2/139 cases (47,XXY and 46,XX,2p+), which most likely did not contributed to the DGS/VCFS phenotype. A 22q11.2 deletion was detected in 17/139 (12.2%) patients tested (14males and 3 females) aged 10days-5 years (n=11), 5-10 years (n=2) and 10-15 years (n=4).In 3 the deletion was inherited (2 maternal and 1 paternal). Patients with 22q11.2 deletion exhibited facial dysmorphic features (82%), congenital heart defects (65%), immunological problems (47%), multiple congenital anomalies including urogenital (41%), hypocalcaemia (35%), mental retardation (35%), behavioral problems (35%), skeletal abnormalities (23.5%), velopharyngeal insufficiency (23.5%) seizures/hypotonia (18%) , cleft palate (18%) and growth retardation 12%). After reevaluation of the remaining patients, 14 were classified under other distinct genetic syndromes which emphasizes the need of stricter clinical criteria for patient referral.

P0078. Partial trisomy 16q in a malformed child with mild retardation

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We report on a 2 year old girl with intrauterine growth retardation, slight dysmorphic features, mild mental retardation and karyotype 46,XX, der(8), t(8;16), (q24.2;q22) mat, confirmed by fluoresent in situ hybridization analysis. The chromosomal anomaly was the result of an unbalanced segregation of a maternal balanced translocation t (8;16) (q24.2;q22). Partial trisomy 16q is a clinically recognized entity with a wide spectrum of anomalies which are compared with our findings. In the literature there are about 40 reported cases, but there are only six previous ones where trisomy 16q is not associated with monosomy of another chromosomal segment, as in our patient. The absence of monosomy as well as the small size of the trisomic segment (16q22→qter)may be the reason of the lack of serious congenital visceral defects in our patient. To our knowledge our report is the first of a partial trisomy 16q resulting from a parental translocation involving chromosomes 8 and 16.This case belongs to a rare group of live born patients reported so far since partial trisomy 16q are usually reported during the 1st trimester.

P0079. Craniosynostosis in a 22q11.2 microdeletion patient without FGFR3 mutation

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Craniosynostosis has been reported in 1 % of the patients carrying microdeletion at locus 22q11.2 which is significantly more common than its incidence in the general population (0.05 %) (Ryan et al, 1997). In the „nonsyndromic" coronal craniosynostosis syndrome (Muencke et al, 1997, MIM 602849) mutation in the FGFR3 gene located at chromosome 4p16.3 is a cornerstone of the diagnosis. In other craniosynostosis syndromes mutations in FGFR1, FGFR2, and TWIST genes were reported. Here we report on a patient with 22q11.2 deletion in whom, among other features, craniosynostosis was also present. The patient, a firstborn of male twins, presenting partial coronal and sagittal craniosynostosis, proptosis, small mouth, intact palate, asymmetric chest interrupted aortic arch, hyperextensible joints, and arachnodactyly, was found to have the common microdeletion of 22q11.2 by FISH (Vysis®). Sequence analysis of the gene FGFR3, and that of genes FGFR1, FGFR2, and TWINS (known as classical craniosynostosis genes) detected no mutations in the hot spot mutation regions. Considering our observation we suggest that the relatively high occurrence rate of craniosynostosis in patients with 22q11.2 microdeletion can not be explained by mutations of the known craniosynostosis genes. We also suggest that the presence of FGFR3 mutation at the locus 4p16.3 in patients with 22q11.2 deletion found in some sporadic observations (Dean et al, 1998, Ryan et al, 1998) might be a pure coincidence of two relatively common mutations.

P0080. Lobar Holoprosencephaly - clinical and evolutional aspects

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

P0081. Phenotype/genotype associations in a 5p- male ascertained for azoospermia.

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The majority of deletions of 5p result in the classical phenotype of the Cri-du-chat syndrome. The associated features include severe mental retardation, microcephaly, growth delay and a cry similar to the mewing of a cat, often the first diagnostic indication for the 5p deletion. Two distinct regions in 5p have been identified by molecular analysis, one responsible for the cat-like-cry in 5p15.3 and the other for dysmorphism, microcephaly, and mental retardation in 5p15.2. A third region for speech delay has been hypothesised in distal 5p15.3. We report on an azoospermic male with 5p deletion, with mild mental retardation, left renal hypoplasia, speech delay, a "breathy, raspy voice", without the clinical features diagnostic for the cri-du-chat syndrome. A cat-like cry was present at birth. He was reported to have had a normal psychomotor development but the very poor performance at school. At the moment he works as warehouseman and is married. Cytogenetic analysis was requested for azoospermia. The deletion breakpoint in the patient, determined by FISH analysis with BAC clones, maps in 5p15.31, 8 Mb far from the telomere. Microsatellite analysis revealed a maternal origin of the deletion. Since most 5p- patients are characterized by severe mental retardation whereas our propositus has only a mild form, this case demonstrates the presence of different mental retardation genes in 5p. The monosomy for that located in the distal 8 Mb is responsible for the mild/moderate type of mental retardation present in our patient. Infertility is not unusual in males carrying chromosome rearrangements.

P0082. Clinical and evolutional remarks on the congenital hydrocephaly

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Objective: diagnostical framing of the echography find out disease, correlation between the imaging and clinical marks, setting of the evolutional stage and therapeutical indication.

Material and Method: The study contained 34 cases of ventriculomegaly, selected by clinical and imaging criteria from the premature newborn hospitalized in the Clinic of Neonatology. Head ultrasonography was used as method of diagnosis and prognosis evaluation.

Results: Hydrocephaly was associated with: meningomyelocele in 8 cases, meningoencephalocele in 3 cases, Dandy-Walker malformation in 4 cases, agenesis of the corpus callosus in 5 cases, malformation of the Galen's vein in 2 cases, arachnoid kyst in 4 cases, lobar holoprosencephaly in 3 cases. In 4 of the cause couldn't be found at they didn't present any associated malformations. 5 of the cases from those with cranio-vertebral dysraphism presented Arnold/ Chiari II malformations associated with meningomyelocele and 3 of those cases presented Arnold/Chiari III malformation associated with meningoencephalocele . The clinical manifestations were those of the classic hydrocephaly associated with: recurring seizures, paresis, paralysis of the inferior limbs in 4 cases, apnea crisis, acrocyanosis. Cerebral imaging was used to appreciate the ventricular enlargement and the degree of compression of the cerebral tissue. The evolution of the cases showed a high rate of decease- 51,4 %.

Conclusions: The fast evoluting hydrocephaly was the primary cause of death at the cases with associated malformations. The most frequently met malformation types were: cranio- cerebral dysraphism and agenesis of the corpus callosum .

Keywords: Hydrocephaly, congenital, child

P0083. The necessity of early positive diagnosis of Dandy-Walker Syndrom

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Introduction: The Dandy-Walker complex corresponds to the Dandy-Walker malformation and to the variants of its syndrom.

Objectives: This work is proposing to frame into the diagnosis the cerebral lesions detected by the antenatal and/or neonatal echography.

Material and method: The study group contains 4 new-borns and infants who were admited in our department between 1998-2004. The criteria for the comprise in the study were anamnestical, clinical and imagistic. Head utrasonography was performed for diagnosis and for dynamic observation of the patients with ventriculomegaly. Physical signs were confirmed by genetics, but without a chromozomial manifestation.

Results: Clinical signs were similar: all the patients presented an increased head circumference, especially for the occipital region. Classical appearance of hydrocefalus was found in only one case. There were present: seizures, hypotonia, apnea and cyanosis and other abnormalities: ASD-one case, congenital hydronephrosis-one case, VSD-one case. The final diagnosis was based on head ultrasonography: enlargement of the IV-th ventricle, maldevelopment of the cerebellum, enlargement of the whole ventriculary system. CT confirmed the diagnosis for all the patients.

The prognosis for the cases was reserved: one patient with evolutive hydrocephalus had a ventriculo-peritoneal shunt at the age of 2 months, 2 patients died at 6 months, one at 7 months.

Conclusions: The diagnosis was set based on imaging studies and clinical evaluation; the associated abnormalities did not influence the prognosis; the hydrocephalus that occure consecutive to the Dandy-Walker malformation evolved fast and caused the death of 3 of the 4 cases.

Key words: malformation, diagnosis, child.

P0084. Remarks on a case of Achondroplasia

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Background: Achondroplasia is a common nonlethal form of chondrodysplasia transmitted as an autosomal dominant trait with complete penetrance. Positive diagnosis is established relatively easy based on specific clinical signs: short stature, rhizomelic shortening of the arms and legs, disproportionately long trunk, trident hands, midfacial hypoplasia, frontal bossing, thoracolumbar gibbus, true megalencephaly and caudal narrowing of the interpedicular spaces.

Material and methods: Premature newborn clinically, imagistic and cytogenetically examined.

Results: A four days old newborn, L.M., has been hospitalised in our department with plurimalformative syndrome. Personal history revealed healthy parents, gestational age of thirtyseven weeks, birth weight : 2260 g, 35 cm stature, Apgar score 1 at 5'. The disease was revealed in the 6th month of the pregnancy. Positive diagnosis has been established based on clinical examination which shows a particular phenotype with: micrognathia, prominent forehead, moderate exophtalmy, posterior cleft palate, micromelia (upper limbs=13cm), short overall stature, siting stature of 30 cm, congenital deformed foot: varus equinus. Paraclinical investigations confirm the clinical data: bone radiography reveales the shortening of the limbs long bones with changes of epiphysis osteochondritis. The genetical consult confirms the existing somatic changes without detecting any change of the karyotype. The evolution in the neonatal period was difficult due to associated intercurrencies (especially repeated respiratory infection), to difficult feeding, with a slowly increasing weight curve.

Conclusions: Achondroplasia is a rare disease in the current medical practice. Diagnosis is easily established in prenatal and postnatal period based on somatometric, cytogenetic and imagistic data.

Keywords:
Achondroplasia, diagnosis, child

P0085. The prevalence of the heart congenital malformations to the premature newborn

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Background: Heart congenital malformations represent an important problem in pediatry because of the growth incidence and of the medical and social implications.

Purpose: This study wants to establish the incidency of heart congenital malformations compared to the other congenital malformations and their frequency according to some factors: risk, social background, sex and prematurity.

Method: The study is based on clinical, paraclinical examinations and imagistic explorations which were performed on premature new born hospitalized in the Neonatology and Health Care Clinic Timisoara between 2000 and 2002.

Results: Of the 72 studied with congenital malformations, 33 (45,8%) had heart congenital malformations. Of these 3 (9%) were cyanotic lesions (transpositions of the great arteries) and 30 (91%) - acyanotic lesions. Regarding the social background 18 (54,5%) were from urban areas and 15 (45,5%) from the rural areas. The repartition of the cases according to sex showed that 16 (48,4%) were female and 17 (51,6%) -male. According to the prematurity: 12 (36,3%) were 1st grade prematures, 12 (42,4%) - second grade prematures, 3 (9%) - 3rd grade prematures and 4 (12%) - 4th grade prematures.

Conclusions: 1. the incidency of heart congenital malformations is high, representing 45,8% of the total congenital malformations.

2. the frequency of the acyanotic lesions is superior to the cyanotic lesions.

3. there is a slight predominance of the cases which come from the urban areas comparatively to those from the rural areas, possibly because of higher pollution.

4. distribution according to sex is approximately equal.

P0086. Phenotype of two siblings with pure partial trisomy 2p21->2p23.

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We present two female siblings at the age of 7 and 3 years, with similar and characteristic findings of short stature (<= 3rd perc.), macrocephaly (> 97th perc.) and mild psycho-motor retardation; a peculiar face with prominent forehead, deep set eyes, blue sclerae, hypertelorism, epicanthus, short philtrum; high arched palate, small teeth, low-set posterior rotated ears with thickened helices, sparse hair, pectus excavatum, clinodactyly of 4th and 5th fingers and broad first toes.
Routine cytogenetic analyses revealed a partial trisomy 2p21->p23, due to a paternaly derived insertion of chromosome 2 material into chromosome 6; karyotype: 46,XX,der(6)ins(6;2)(p12;p21p23)pat. These results were confirmed by reverse in situ hybridisation.
In contrast to terminal trisomy 2p that is repeatedly reported, interstitial partial trisomy 2p is rarely observed. The delineation of phenotypic findings to causative, non-terminal partial trisomy 2p is puzzeling. Up to now, there is only one publication of the same partial trisomy 2p21->p23. Clinical features in common are: growth failure, macrocephaly, development delay, prominent forehead, low-set ears with helix anomaly, and clinodactyly. A comparison with other cases of partial trisomy 2p extending beyond 2p21->p23 confirms these observations. Our studies now allow to delineate a common phenotype of trisomy 2p21->p23 and lead to a more accurate genotype-phenotype assignment in partial trisomy 2p.

P0087. Supernumerary interdigital flexion creases as marker of monosomy 21q22.3, an underdiagnosed cryptic deletion syndrome

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There is a broad variation in the clinical features of distal q21 deletions. Patients with monosomy 21q22.3 are rarely described. Therefore we report a girl karyotyped with cryptic monosomy 21q22.3 and without evidence of mosaicism. The diagnosis was confirmed by subtelomer FISH analysis. Our patient was referred because of global developmental delay and minor morphological anomalies. Her weight, length and head circumference is normal. The girl has a mild midfacial hypoplasia and abnormalities in sleeping EEG as distinctive features. A wide range of midline facial abnormalities with or without pathological EEG can be associated with holoprosencephaly which is inter alia related to deletion of chromosome 21q22.3. Comparison with patients with large ring chromosome 21 revealed a similar recognizable phenotype with multiple supernumerary interdigital flexion creases without digital abnormalities as a characteristic marker for this aberration. This particular phenotype was found to be present bilaterally on the middle phalanges of the fingers I-IV in our patient. Multiple supernumerary interdigital flexion creases are rare in the general population, but occur frequently in a number of syndromes such as Alagille syndrome, which is caused by haploinsufficiency of the JAG1 gene, located at 20p13. Therefore another haploinsufficient gene leading to this rare morphological anomaly in a syndromal context seems to be located at 21q22.3.

P0088. Williams Syndrome - an Epidemiologic Approach

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Williams syndrome (WS) is a microdeletion syndrome that is characterized by distinctive facial features, cognitive impairment, unique personality characteristics, cardiovascular anomalies, growth deficiency and infantile hypercalcemia. The mainstay for diagnosis is the contiguous gene deletion in the WS critical region (located at 7q11) that encompasses the elastin gene. Clinical diagnosis is confirmed using Fluorescent In Situ Hybridization (FISH).
We propose to analyse the frequency of WS in Cyprus, a small island in the eastern Mediterranean with a population of 700,000. This population is served by a relatively new genetics clinic which was established in 1994. We discuss the clinical variability of this disorder among the patient population in our clinic. We will also attempt to correlate the effect of early intervention on the natural history of WS. The incidence of WS in the Cyprus is approximately 1% of the patients seen in the clinic. The diagnosis was established as early as a few months and as late as 16 years. Many of the patients had the classic clinical picture; there were however a few who did not have the expected spectrum of features (congenital heart disease, infantile hypercalcemia or growth deficiency).
We suggest that the patients in Cyprus have a greater heterogeneity with a clinical picture at the two ends of the WS spectrum. Even though we had the opportunity to offer early intervention we found that it was not a single influencing factor in the cognitive development of children with WS.

P0089. Should chromosome breakage studies be performed in patients with VACTERL syndrome ?

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VACTERL is characterized by the association of vertebral anomalies, anal atresia, cardiac malformations, tracheoesophageal fistula, renal anomalies, and limb anomalies and is postulated to be a very heterogeneous disorder. Those malformations are all part of the Fanconi anemia spectrum. Although the association of VACTERL with hydrocephaly have clearly been associated with Fanconi anemia in several instances, the indication for chromosome breakage studies is not clear in VACTERL without hydrocephaly. Here we report on 5 unrelated patients with VACTERL and FA. Together with the data of 13 VACTERL/FA cases extracted from a large previous European genotype-phenotype correlation study and those from the 3 reported cases in the literature, we showed that: i) the frequency of VACTERL association in FA patients can be raised at 13/243 (5%), ii) although at least one other feature of FA was found in the vast majority of cases (café au lait spots, growth retardation, microcephaly, dysmorphism), none of those feature was found in 2 cases (10%), and iii) VACTERL phenotype in FA appear rare in the more prevalent groups A, C and G and frequent in complementation groups D, E, F (>30%). 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, not only in cases of VACTERL-H, but also in VACTERL, especially if the child also present skin pigmentation abnormalities, growth retardation, microcephaly or dysmorphism.

P0090. Genotype-phenotype correlations in X-linked Periventricular Nodular Heterotopia

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In PNH (Periventricular Nodular Heterotopia) subsets of neurons fail to migrate to the developing cerebral cortex and remain bordering the lateral ventricular walls.
Classical PNH, an X-linked dominant disorder is far more frequent in females, who have normal intelligence to borderline mental retardation, epilepsy and extra-CNS signs. Prenatal or early postnatal lethalithy in males was observed in most pedigrees.
The disorder is associated with mutations in *Filamin A* gene (*FLN1*) localised to Xq28 and coding for the 280 kDa protein *Filamin A*. In order to define genotype-phenotype correlations, we performed clinical examination, cognitive testing, MRI and *FLN1* molecular analysis (by using DHPLC and direct sequencing) on 89 patients with PNH.
22 probands (38 patients including familial cases) carried different mutations (either missense, nonsense or missplicing) throughout the *FLN1* gene. These concerned 9/10 (90%) pedigrees consistent with X-linked inheritance and 13/80 (16%) of sporadic cases.
Most patients had bilateral contigous heterotopic nodules while a few carrying missense mutations had unilateral isolated or non contigous nodules. None of the patients with periventricular nodules harboured mutated *FLN1*. We also identified male patients who carried missense or mosaic truncating mutations, one transmitting the disorder to his daughter.
In conclusion, mutations of the *FLN1* gene account for 90% familial cases with clear X-linked inheritance and concern both genders, although they are clearly predominant in females. Our results confirm that *FLN1*, is the main gene associated with X-linked PNH although they support the implication of other genes.

P0091. Spontaneous recovery of a childhood-onset mitochondrial myopathy caused by a stop mutation in the mitochondrial cytochrome c oxidase III gene

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Recently, we reported on a patient suffering from mitochondrial myopathy with ragged red fibers (RRF), lactic acidosis, exercise intolerance and delayed growth with a heteroplasmic G9379A nonsense mutation (W58X) in the mtDNA encoded *COIII* subunit gene. An actual follow up examination of the patient showed remarkable clinical and electrophysiological improvement. On a second muscle biopsy signs of histological and immunohistological improvement of the mitochondrial myopathy were found, which was associated with a significant decrease (from 93% to 50%) of the mutational load of G9379A in skeletal muscle confirming a spontaneous regression of the disease. Myoblasts of the patient did not carry the mutation, therefore we suggest that the fusion of wild-type mtDNA containing myoblasts into existing muscle fibers might positively influence the mutational rate in our patient´s muscle. Our results demonstrate the variable course of diseases caused by mtDNA mutations. We suggest that this possible positive outcome should be considered in counseling some patients with mtDNA mediated disorders.

P0092. Somatic mosaicism of the *FLN1* gene in patients with periventricular nodular heterotopia

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Periventricular nodular heterotopia (PNH) is a malformation of neuronal migration in which a subset of neurons fails to migrate into the developing cerebral cortex and remains as nodules of heterotopic gray matter along the lateral ventricles.
X-linked PNH is associated with mutations of *Filamin A (FLN1)* gene (mapped to Xq28) and is characterised by 50% recurrence risk in daughters of affected women and prenatal or early postnatal lethality in boys. Partial loss-of-function of *FLN1* or somatic mosaicism were hypothesized to explain viability in two men with PNH recently described.
We have performed mutation analysis (DHPLC and direct sequencing) and Single Nucleotide Primer Extension (SNuPE) associated with DHPLC on two families in which probands carried *FLN1* mutations in a mosaic fashion.
In the first family, an affected man with classical PNH was mosaic for an A>G substitution (intron 11 acceptor splice site) on both leukocyte DNA and hair roots. Single hair root analysis confirmed that the mutation was not present in all ectodermal derivative cells. His daughter had inherited the X chromosome from her father's wild type germinal cell population.
In the second family, a woman with mild features of PNH was mosaic for a nucleotide insertion (c568_569ingG). The somatic mosaicism could explain the phenotype of this woman that was less severe than expected for a frameshift mutation.
Somatic mosaicism is one of the genetic mechanisms that may explain phenotypic variability in patients with *FLN1* mutations. Mosaicism investigation using DHPLC should support genetic counselling in patients with PNH.

P0093. Down syndrome associate with Hirschprung disease

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Introduction: Frederick Ruysch describes for the first time in 1691 a child with "Enormi intestine dilatation coli". It has been more than 120 years since Harald Hirschprung characterized congenital megacolon in Berlin but the etiology remain unknown.
Purpose: The aim of the study is to evaluate the incidence and complications occurred in Hirschprung disease associated with Down syndrome.
Material and methods: We reviewed the medical records and cariotype examinations for the 68 patients diagnosed and treated for severe constipation in the last 20 years (1984-2003) in Department of Pediatric Surgery, Timisoara. All the patients diagnosticated with Hirschprung disease were operated by Duhamel procedure.
Results: Fourteen patients presented: meconium ileus (7pts), intestinal atresia (2pts), intussusception (1pts) and secondary megacolon (4 pts) and were excluded from the study. Fifty-four patients present Hirschprung disease based on clinical findings, X-ray barium enema, rectal biopsy and imunohistochimic studies. Three patients (5, 5%) present Down syndrome based on cariotype examination on 15 mitoses.
Postoperatory results were very good in forty-five patients (83, 3%) good in seven patients (12, 9%) and satisfactory in two patients (3, 7%). Septic complications occurred in four patients (7, 4%) two of them with Down syndrome.
Conclusions: The 5, 5% patients with Down syndrome represent the four folded incidence compared with normal population. It is not clear until now if the genetic defect in megacolon result in ganglion migration defect or in environment problems.
Association between Hirschprung disease and Down syndrome determine a higher rate of complications and implies a worsen prognosis.

P0094. First description of coumpound heterozygosity for the 657del5 and R215W mutations in the NBS1 gene in severely affected twin brothers

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Nijmegen breakage syndrome (NBS), an autosomal recessive chromosomal instability disorder, is clinically characterized by congenital microcephaly and facial dysmorphology, immunodeficiency, combined with hypersensitivity to ionizing radiation, an increased risk for lymphoreticular malignancy. Most NBS patients are of Slavic origin and homozygous for the founder mutation 657del5. The frequency of 657del5 heterozygotes in the Czech population is 1:150. Recently, another NBS1 mutation, R215W, of unknown pathogenetic relevance, was found to have a higher frequency among tumor patients of Slav origin than in the control population. This alteration results in the substitution of a basic (arginine) with a nonpolar amino-acid (tryptophan) and thus could inactivate some nibrin functions.
We report for the first time on two monozygotic twin-brothers who are compound heterozygotes for the 657del5 and R215W mutation. Both children showed chromosomal instability. They were born hypotrophic at the 33rd week of pregnancy with premature closure of fontanells suture. Poor gyrification and enlarged lateral ventriculs were shown by ultrasound investigation of the brain. At the age of 5 weeks, OFC, weight, and length were all under the 5th percentile. At the age of 5 months they were treated for status epilepticus and their psychomotoric development was delayed. At the age of 10 months they developed microtrigonoturricephaly, muscle hypotonia, and severe respiratory and feeding problems. We hypothesize, that the compound heterozygote status of these boys is the primary cause of their severe clinical and cellular phenotype. As a consequence, we postulate that homozygosity for the R215W mutation will lead to a disease phenotype.

P0095. The features of clinical manifestation of different types of Prader-Willi syndrome.

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Prader-Willi syndrome (PWS) is a paradigm for epigenetic imprinting. Functional nullisomy of the PWS genes (15q11-q13 region) results from normal. Selective inactivation of paternally inherited alleles is caused by deletion, maternal uniparental disomy (UPD15mat), or mutations in imprinting control region on paternal chromosome 15. The specific of clinical manifestation of different forms of paternal alleles inactivation are not understood entirely. We report about 3 groups of patients with clinical features of the PWS. First group (n = 31) was presented by probands with 15q11-q13 deletions detected by FISH-analysis. Second group (n = 13) of patients had UPD15mat. In third group of patients (n = 13) DNA methylation analysis did not revealed any abnormalities specific to PWS. 64 clinical symptoms classified to 12 groups of development abnormalities were scored. Correspondence analysis has revealed a significant difference between 3 groups (χ^2=156.27; P=0.014). Congenital hydrocephalus and round face were typical for probands with deletion form of PWS. Diastem was characteristic for probands with UPD15mat. Individuals with clinical features of PWS but normal DNA methylation status were distinguished by a high tall, increased frequency of microstomia, congenital epicanthus, aortic septal defect and decreased rate of hypothyroidism. We didn't show statistical differences between groups of patients with deletion or UPD15mat (χ^2=41.10; P=0.38), but probands with deletion were characterized by increased frequency of anomalies of spine (χ^2=6.69; P=0.009), extremities (χ^2=7.08; P=0.007) and nervous system (χ^2=13.69; P=0.002). Our results indicate UPD15mat had a milder effect to phenotype than deletion of imprinted genes on chromosome 15.

P0096. First report on congenital prosopagnosia outside the caucasian population

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Prosopagnosia or face blindness is defined as the inability to associate a face to a person, while faces as such and facial expressions can be recognized. The specifity of this deficiency is best supported by double dissociation between probands with impairment of e.g. object agnosia but not prosopagnosia and vice versa. Almost all reports (medline n < 400) are single casuistics or collections of unrelated patients who acquired prosopagnosia after brain injuries, strokes or atrophy of at least the right occipito-temporal cortex.
In contrast, the inborn form was described only in about a dozen probands of caucasian origin.
We recently found that congenital prosopagnosia has a very high prevalence of 1 - 2 %, and that it is genetically determined - best described by autosomal dominant inheritance in all 20 families ascertained (Kennerknecht et al. (2003) Eur J Hum Genet 10 (Suppl. 1): 249). The high frequency in the caucasian population prompted us to extend our search for other ethnic groups. We performed a questionnaire based screening among 160 native indian students at Banaras Hindu University in Varanasi. In a then selected subset we found after further detailed diagnostic interviews one Bengali female student with visual agnosia for face recognition only.

P0097. Congenital Nasal Pyriform Aperture Stenosis, Associated Anomalies And Syndromes

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Congenital nasal pyriform aperture stenosis (CNPAS) is a rare cause of congenital nasal airway obstruction, requiring surgical intervention. It has been reported in holoprosencephaly (HP) cases and can also occur as an isolated finding or in association with other systemic anomalies. Several chromosomal anomalies have been described with CNPAS. We aimed to describe the whole phenotypic spectrum and prevalence of chromosomal and/or additional congenital anomalies associated with CNPAS. 31 unrelated patients aged 3.5±3.2 years had dysmorphological evaluation, pituitary function assessment, brain MRI, ophthalmologic, vertebral, heart, kidney and chromosomal investigations. A family history of midline defect was found in 5 (17%) and parental consanguinity in 8 (26%). Of the 31 patients, 25 (81%) had additional congenital anomalies including: solitary median maxillary central incisor (SMMCI) (55%), brain malformation (60%) (none had holoprosencephaly), ophthalmologic anomalies (40%), cardiac malformation (22.5%), vertebral segmentation defects (18%), radial ray (13%) and renal anomalies (10%). 8 of them (26%) had pituitary hormone deficiencies and/or abnormal hypothalamo-pituitary MRI finding (39%). Among those patients we identified 6 known associations: 4 VACTERL, 1 CHARGE, 1 RHYNS syndrome. Only one child had a chromosomal anomaly (45,X). The genetic basis of CNPAS and its relation with HPE are still unknown, but it is likely that a number of mechanisms can give rise to it. Additional malformations or endocrine abnormalities are frequently associated and justify complete evaluation in affected newborns for appropriate treatment and genetic counselling.

P0098. The study of Marfan syndrome in Republic of Bashkortostan

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Marfan syndrome is an autosomal dominant disoder of connective tissue. Despite the fact that frequency of the disease in the world is 1 to 5000, this ratio is in the Republic of Bashkortostan is 0,1 to 5000. Such a low ratio is probably associated with lack of effective medical and genetic consultation of the population living in remote populated areas: 83% of the patients live in large cities. More than a half of 90 patients who are under prophylactic medical examination in connection with Marfan syndrome represent sporadic cases.

The group under examination is characterized by mild forms of the disease while neonatal and atypical severe forms of the Marfan syndrome are absent.. The aim of our research is to investigate genetic and epidemiologic base of Marfan syndrome and work out methods of DNA-diagnostics of the disorder. To date we analyze DNA samples of 52 probands. In order to detect mutations we use SSCP method, hetroduplex analysis with further direct sequencing. By present time we have carried out SSCP analysis of eight exons and direct sequencing of two exons of the FBN-1 gene in 36 probands. In one case we found polymorphism 2161-46a/g in intron codon adjacent to the18th exon of the FBN-1 gene. In another case we found polymorphism in 24 exon (984V/V). In the third case a polymorphism 1057S\S in 25 exon was found. The screening of mutations in other exons of the FBN-1 gene is still carried on.

P0099. Progressive osseous heteroplasia associated with a de novo mutation of GNAS1 gene.

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Heterotopic bone formation in skin and subcutaneous tissues (osteoma cutis) is rare and can be primary or secondary to tumors, traumatic and post-inflammatory events. Primary osteoma cutis can be associated with several genetic disorders among which fibrodysplasia ossificans progressiva (FOP), Albright's hereditary osteodystrophy and progressive osseous heteroplasia (POH).
POH is a rare autosomal dominant disorder, characterised by dermal ossification in infancy followed by progressive and widespread ossification of skin and deep connective tissues. Paternally inherited heterozygous inactivating mutations in the gene encoding the alpha subunit of the stimulatory G protein of adenylyl cyclase (GNAS1) have been identified in POH patients.
We report on a two year-old girl presenting with neonatal and progressive osteoma cutis lesions with poor possibility of movements, pre- and post-natal severe growth deficiency, left hemihypotrophy, infiltrating myocardial tumors and cystic dysplasia of the right kidney. Calcium, phosphorus, parathyroid hormone and Gs alpha protein activity levels were normal, as well as blood chromosomes.
Althought this atypical clinical presentation, we performed GNAS1 molecular analysis, which revealed a de novo 344-345insT frame-shift mutation in exon 5, previously described in a POH patient (Shore et al., 2002).

P0100. The Results of Cytogenetic Analysis in 150 Autistic Patients

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The autistic disorder was first described by Leo Kanner sixty years ago. This complex developmental disability is characterized by social and communicative impairments, repetitive and stereotyped behaviours and interests. The prevalence of autism in the general population is about 1 in 1000 with four males affected for one female. In approximately 15% of the cases, autism is associated with known genetic disorders.
Nearly one third of the specific genetic causes of autism are due to chromosomal disorders. They account for less than 5% of all cases of autism, although this estimate varies.
We performed a detailed genetic analysis of 150 autistic patients focused on cytogenetic investigation. In our cohort, 7 subjects were identified as having chromosome anomalies (4.7%). One patient exhibited a de novo balanced translocation, 2 patients inherited a balanced chromosomal translocation from their mother, 1 patient had trisomy 8 mosaicism, 1 patient exhibited mosaicism with ring chromosome 17, 1 had inv dup (15) chromosome and 1 patient exhibited del (17) (p11) and the Smith-Magenis syndrome. The quite high frequency of chromosomopathies supports the hypothesis that PDDs may develop as a consequence of chromosomal abnormalities, and justify the cytogenetic examination in all patients with PDDs for establishment of their diagnosis.

P0101. Sebaceous Nevus syndrome with spinal neurofibroma: A new contiguous gene gyndrome?

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Sebaceous nevus syndrome (SNS) is a distinct neurocutaneous disorder consisting of sebaceous nevus, typically localized to the scalp and face, and a broad spectrum of neurologic, ophthalmologic, cardiovascular, urogenital and skeletal findings. Affected individuals are at increased risk of developing both intracranial and extracranial tumours. NF-1 Microdeletion Syndrome is a newly recognised condition involving clinical features of NF-1, dysmorphic features, and congenital heart disease. We present here a newborn girl with a facial sebaceous nevus, dysmorphic features, seizures and coarctation of aorta. MRI of the brain and spine revealed a large mass extending from C1 to C6, with a small enhancing lesion anterior to the pons. Needle core biopsy of the spinal mass showed it to be consistent with a neurofibroma, with radiologic features suggestive of a plexiform neurofibroma. The patient had no other features of NF-1. The family history was non-contributory. Neurofibromas are not a well-recognised feature of SNS. Courville et al. (2000) reported a patient in whom a plexiform neurofibroma was mixed with a large epidermal nevus. Booth and Rollins (2002) reported two cases of SNS, with spinal lipomas and abnormally enhancing, enlarged spinal nerve roots on MRI. These findings have been seen in some patients with NF-1. These cases suggest a possible link between NF-1 and SNS, raising the question of whether the phenotype in the present case may represent a contiguous gene syndrome involving the NF-1 gene and the unknown SNS gene(s). We are performing FISH analysis in this region and will present our findings.

P0102. Branchio-Oculo-Facial (BOF) syndrome in two infants: additional help required.

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BOF syndrome consists of branchial defect, dysmorphic face (dolichocephaly, sparse hair, high forehead, malar hypoplasia, small chin), ocular anomaly (asymmetry, coloboma, microphtalmia), wide nasal bridge, prominent nose, abnormal upper lip and philtrum, some type of cleft (lip, palate, pseudocleft) and renal anomaly. Pre-, postnatal growth retardation, developmental and mental delay are associated findings. Presence of thymic remnant in cervical tissue could be characteristic. Autosomic dominant inheritance was postulated but no causal gene was identified so far. Mutations in Eya1 gene - implicated in branchio-oto-renal (BOR) syndrome- were not found in BOF syndrome. We present two unrelated infants with probable BOF syndrome born from non-consanguineous healthy parents. Both patients developed the above described dysmorphism, microphtalmia, feeding difficulty and hypotonia but no hemangioma. Patient 1 presented with pseudocleft, right branchial mass and an unilateral pyelo-ureteral junction stenosis. The histology of the entire cervical lesion failed to identify thymic remnant (branchial cyst). Patient 2 presented facial asymmetry, left renal agenesis and hypoplastic C3, C4 vertebral bodies without vertebral defect. Complex inner ear anomaly and deafness were recorded in patient 1 but not in patient 2. The standard karyotype was normal. No 22q11.2 deletion was found. Eya1 gene mutation was not found in patient 2. Because genetic heterogeneity exists in BOR syndrome and due to absence of definite marker in BOR and in BOF syndromes, question remains on a continuum disease rather than distinctive entities. Additional identification and comprehension on genes and mechanisms implicated in first and second arch development are needed.

P0103. Autosomal dominant Alport syndrome: natural history of a disease due to COL4A3 or COL4A4 gene.

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Alport syndrome is a clinically and genetically heterogeneous nephropathy. The majority of cases are transmitted as an X-linked semi-dominant condition due to COL4A5 mutations. In this form males are more severely affected than females. Less than 10 % of cases are autosomal recessive due to mutation in either COL4A3 or COL4A4. In this rarer form, both males and females are severely affected. Only two cases of autosomal dominant Alport syndrome have been reported, one due to a COL4A3 mutation and the other due to a COL4A4 mutation. Because of the paucity of the reported families, the natural history of autosomal dominant Alport syndrome is mostly unknown. We investigated four families with likely autosomal dominant Alport syndrome. The COL4A3 and COL4A4 genes were analysed by Denaturing High Performance Liquid Chromatography. Automated sequencing was performed to identify the underlying mutation. Two families had a mutation in the COL4A4 gene and two in the COL4A3. Accurate clinical evaluation of family members showed interesting results. Affected individuals (22 persons) had a wide range of phenotypes from end-stage renal disease in the fifth decade to a non progressive isolated microhematuria. Finally, three heterozygous individuals (90, 22 and 11 years old, respectively) were completely asymptomatic. Our results demonstrated that patients affected by autosomal dominant Alport syndrome have a high clinical variability. Moreover, a reduced penetrance of about 90% (3/25) may be considered for the assessment of recurrence risk during genetic counseling of these families.

P0104. The evaluation of an infertile family with Y chromosome microdeletion

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Microdeletions of the so-called azoospermia factor (AZF) locus of the Y chromosome long arm (Yq) have been recognized as an etiological factor of severe oligozoospermia or azoospermia. In general, men with deletions are infertile and therefore deletions are not transmitted to sons unless in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are performed.
We report a family characterized by multiple members with infertility and Yq microdeletion. Complete reproductive history, semen analyses and blood samples were elicited from relevant family members. DNA preparation and quantification were performed using commercial kits. A total of 15 pairs of sequence tagged sites based primer sets specific for the Y microdeletion region loci were used for screening. We report the case of an azoospermic patient (proband) who inherited an extensive Yq microdeletion from his father through a spontaneous pregnancy. The proband‘s father was a sevety-yr-old man who had 3 other children, 2 males and 1 female. The proband, his father and the other 2 brothers carried a Yq microdeletion of the AZFb subregion. The proband also carried Yq microdeletion on AZFa and AZFc region. The proband, his two brothers and father were all found to be deleted for RBM. At the time of analysis, the proband‘s brothers were azoospermic. Unlike their father, the all three sons are infertile and have no offspring.
Microdeletions of Yq involving the RBM gene are associated with a variable phenotypic expression that can include evidently normal fertility.

P0105. Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). A threshold effect of urinary glycosaminoglycans and the walk test as indicators of disease progression in a survey of subjects with mucopolysaccharidosis VI (Maroteaux-Lamy syndrome)

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A cross-sectional survey in individuals affected with the lysosomal storage disease Mucopolysaccharidosis VI (MPS VI) was conducted to establish demographics, urinary glycosaminoglycan (GAG) levels, and clinical progression of the disease. The survey evaluated 121 *bona fide* MPS VI-affected individuals over the age of 4 years from 15 countries across the Americas, Europe and Australia representing greater than 10% of the estimated world prevalence of the disease. A medical history, complete physical exam, urinary GAG determination, and assessment of several clinical measures related to endurance, pulmonary function, joint range of motion and strength, and quality of life were completed for each participant. Although a wide variation in clinical presentation was observed depicting MPS VI as a clinically heterogeneous disorder, several general findings were obtained reflecting progression of the disease. Impaired endurance, as measured by the distance achieved in a 6-minute walk, could be demonstrated across all age groups of MPS VI affected individuals. High urinary GAG values (>200 µg/mg creatinine) were associated with a more accelerated clinical course based on age-adjusted short stature and low body weight, impaired endurance, compromised pulmonary function, and reduced joint range of motion. An unexpected result was a dramatic cut-off of urinary GAG levels at approximately 100 µg/mg creatinine for participants over the age of 20 years. These results suggest that urinary GAG levels predict clinical morbidity, and longer-term survival is achieved in subjects whose GAG level is below the threshold of 100 µg/mg creatinine.

P0106. Trisomy 20 mosaicism: the first case not ascertained after prenatal detection of the mosaicism

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Trisomy 20 is one of most common forms of mosaicism at prenatal diagnosis. 90% of the born children are grossly normal and exhibit normal development; 10% have serious defects. In these cases trisomic cells are usually present in the fibroblasts but not in the blood. We report the first case of trisomy 20 mosaicism in a girl with dysmorphism and congenital malformations. She was born from a 44-years-old mother; birth weight and length were at 50th centile. Clinical examination at one month of age revealed hypotonia and growth deficiency, congenital scoliosis and vertebral anomalies, hypoplastic iliac bones, 13 pairs of ribs, large forehead, epicanthic folds, deep philtrum, low posterior hairline, labia majora fistula, large clitoris. At1 year of age she was operated for a hiatal hernia after melena and at 4 years she had punctate vasculitis skin lesions.
Now, at 10 years, she presents normal mental development, no dysmorphism , severe scoliosis; pelvic and abdominal ultrasounds showed bicornuate uterus and small right kidney. Examination of the skin showed streaky hyperpigmentation on the thorax, upper and lower arms.
Repeated lymphocyte chromosome analysis detects a normal karyotype. At the last observation, the presence of the skin pigmentation abnormalities prompted the geneticist (SG) to request a chromosome analysis from fibroblasts detecting 27 out 30 cells with trisomy 20. FISH analysis confirmed this finding. Microsatellite

analysis revealed the maternal origin of the trisomy 20 excluding the uniparental disomy. This case stresses the importance to investigate skin fibroblasts in skin pigmentation anomalies.

P0107. An additional case of the KBG syndrome

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We report the case of a child who was sent for genetic diagnosis on the association of growth retardation, facial dysmorphism and mental retardation. Pregnancy had been complicated by oligoamnios, unique ombilical artery and severe intrauterine growth retardation. His mother suffered from a unilateral hearing loss and her height was at -1,5 SD. The patient was born prematurely by caesarean section at 29 GW (BW: 650g, BL: 31 cm, BHC: 24.8 cm). At 4 years of age, the boy showed growth delay (-2SD) and a characteristic facial appearance including broad forehead, hypertelorism, strabismus, slight mongoloid slanting of the palpebral fissures, marked anteversion of the nostrils, and retrognathia. Lips were thin and the upper lip was shaped like a hunter's bow. He had malpositionned medial superior teeth and oligodontia. His hands were short. The subcutaneous fatty tissue was not much developed and he had "café au lait" spots on his back, his hair was thick. He had severe developmental delay with speech defect and autistic features. He suffered from night myoclonies and partial seizures.
Investigations showed a pelvic left kidney on the intravenous urography, enlarged sub-arachnoidal spaces on brain MRI, brachycephaly and fused C2-C3 vertebral bodies on the skeleton roentgenogram. Lymphoblastic and fibroblastic karyotypes were normal : 46,XY
All those features are compatible with the KBG syndrome, an autosomal dominantly inherited syndrome, first described by Herman and al in 1974. Only 27 cases have been reported so far.

P0108. Genotype-phenotype correlations in 177 patients with autosomal recessive polycystic kidney disease (ARPKD)

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Autosomal recessive polycystic kidney disease (ARPKD) is associated with PKHD1 mutations on 6p12. ARPKD is characterized by renal cysts of tubular and collecting ducts and congenital hepatic fibrosis (CHF). While the majority of cases manifests peri-/neonatally with a high mortality rate in the first month, the prognosis of surviving patients is better. This is the first study that reports the long-term outcome of ARPKD patients with defined PKHD1 mutations. We examined the clinical course of 161 neonatal survivors (126 unrelated families) over a mean observation period of 6.0 years (0-35 y). The 1- and 5-year survival rate was about 95%. The median age of SCr values of >2 SDs was 5.1 years. All but six patients (92%) had a kidney length above or on the 97th centile for age. About 80% of the study population developed systemic hyper-tension. Around 40% of patients presented with clinical signs of CHF (splenomegaly, oesophageal varices, or ascites). Manifestation of CHF was positively related with increasing age. Significant correla-tions could be further demonstrated between renal- and hepato-biliary-related morbidities sugge-sting uniform disease progression rather than organ-specific patterns. PKHD1 mutation analysis revealed a total of 190 mutations, among them 70 novel ones. None of our patients carried two truncating mutations corro-borating that one missense mutation is indispensable for survival of newborns. We set up genotype-phenotype correlations and catego-rized missense mutations. The obtained mutation detection rate of 75.4% with more than 95% of patients with at least one mutation makes PKHD1 mutation screening robust for clinical use.

P0109. Prader-Willi syndrome - the newborn dysmorphic phenotype is a reliable guide for early diagnosis

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Prader-Willi syndrome (PWS) is a genetic disorder characterized by obesity, acromicria, mental retardation and hypogonadism.
In neonatal age PWS is expressed differently, mainly by severe muscular hypotonia and feeding difficulties. Some authors have described neonatal presentation of PWS, but most of them have not focused on distinctive craniofacial features and have not presented photographs. We introduce here 4 newborns with PWS diagnosed during 1st month of age and present their photographs.
All four patients (3f/1m) were born at term. Two of them had low birth weight (2316g and 2700g) and cephalhaematomas. Soon after birth all patients showed apathy or lethargy, central muscular hypotonia, almost absent weak cry, no interest to food, aided feeding and termolability. Their faces were hypomimic with insufficient opening of the eyes. They had distinctive facial features, high prominent forehead, narrow bifrontal diameter, down-turned corners of the mouth, micrognathia and dysplastic ears. The last case was recognized immediately due to the similarity with the third one. In one patient the PWS was caused by 15q11-13 microdeletion, in one by unbalanced 14;15 translocation causing 15q11-13 microdeletion and in two by maternal UPD15.Four years ago we started systematic search for PWS patients in Estonia. Attention to the complex clinical features during the neonatal period resulted in the detection of 4 PWS cases in newborn age. In our opinion the face of infants with PWS is distinctive and the combination of neonatal hypotonia, feeding difficulties and distinctive facial features is specific for recognizing PWS in neonatal age.

P0110. Homozygous patients for a CCTG repeat expansion in a family with Myotonic Dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is caused by a dominantly transmitted CCTG repeat expansion in intron 1 of the zinc finger protein 9 (*ZNF9*) gene on chromosome 3q. The clinical symptoms resemble those of DM1, but the disease course is milder and no mental deterioration is observed. A congenital form is lacking. DM2 patients with two mutant alleles are not reported so far. In one large consanguineous family from Afghanistan we found three homozygotes for the CCTG repeat expansion. The oldest patient was clinically most severely affected, compared to the two younger homozygotes, but for the clinical course of symptoms all three homozygotes were within the range expected for heterozygotes. Further investigations, such as repeat length, muscle histology, muscleblindlike 1 (MBNL1) stainings or brain imaging studies, at least at short-term observation showed no differences between heterozygotes and homozygotes. Eleven children in the third generation, aged 2 to 21 years, harbouring a repeat expansion, were available for clinical exam and genetical analysis. None of these heterozygous children have signs or symptoms of disease till the age of 18 years. At present, our analysis indicates, that homozygosity for the DM2 mutation does not severely alter the ability for a normal pregnancy and birth, the age of onset of symptoms, the phenotype, the rate of disease progression or anticipation.

P0111. A case with Leigh syndrome and SURF1 gene mutations

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Mitochondrial cytopathies are clinically and biochemically heterogeneous disorders affecting energy production. Leigh syndrome is one of the most common disorders of mitochondrial energy generation presenting in childhood. The classic clinical features include progressive neurological disease with motor and intellectual developmental regression, symptoms of brainstem or basal ganglia disease, lactic acidosis, and characteristic neuropathological features on cerebral imaging. Leigh syndrome can be caused by a number of enzyme defects including cytochrome c oxidase (COX) deficiency. Mutations in the SURF1 gene were recently shown to be a common cause of COX-deficient Leigh syndrome

We present a 3 year-old girl born at term from second normal pregnancy and delivery. No consanguinity and family history were found. She had no problems until one year of age when growth retardation (SDS-3), generalized hypertrichosis and unstable walk were noticed. During an intercurrent illness we found severe lactic acidosis associated with hypotonia, movement disorders and cerebellar ataxia.

Urine organic acid analysis excluded organic acidemias, plasma amino acids were normal. Neuroradioimaging procedure showed bilateral symmetrical hypertense signal abnormality in basal ganglia on T2-weighted magnetic resonance imaging. Echocardiography was normal. The child is with normal intellectual development. Biochemical analysis of muscle biopsies detects deficient activity of the IVth respiratory chain enzyme complex (COX). In skin biopsy was found that the child is compound heterozygous for two mutations in the SURF1 gene: 312del10insAT in exon 4 and 845delCT in exon 9.

P0112. Adrenoleucodystrophy and epilepsy

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An paroxysmal disorders of consciousness in adrenoleucodystrophy (ALD) in the majority of clinical observing present from orthostatic and cardiogenic syncopes. In the base “Medline” for 1981-2004 does not be kept information on epileptic sezures at ALD patients.

Material: observation of adolescent with the combination ALD and cryptogenic epilepsy.

Objective: practically healthy boy at 10 years stopped in psychomotor development, then the cognitive disorders increased. In 13 years darkening of the skin has developed, hyperpigmentation of nipples and genitalia. From 14 years the patient has monthly primary-generalized tonic-clonic epileptic seizures, which are appearing after arousing or falling asleep. At the checkup in 16 years the local neurological signs are absent, the subcortical dementia is revealled, psychomotor disorders too. Cerebral T2-weighted MR demonstrates confluent, symmetrical, hyperintense lesions of the white matter bilaterally. Adrenals on RCT reduce in size, cortex is thin. In blood analyses: ACTH 377,8 pmol/l (N - 2,2-13,4), cortisol 144,0 nmol/l (N - 190-650). In t urine analyses: general cortisol 228 nmol/day (N - 300-750). EEG: diffuse disorders of electrical activity, epileptiform activity not revealled. Treatment: substitute glucocorticoid therapy. Never got of systematic antiepileptic treatment. After starting Depakine Chrono 1500 mgs a day therapy the sezures are stopped, but the phenomenas of adrenal insufficiency and dementia are increasing.Conclusion: Synthetic ACTH analogues are used as antiepileptic drugs in some forms of epilepsies. Possible, hyperproduction of ACTH is explained the rarity of epileptic sezures at ALD. However, it is necessary to take a possibility of presence of epilepsy.

P0113. Croatian girl with Angelman syndrome due to UPD - the evidence of genotype-phenotype correlation

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Background: The Angelman syndrome (AS) is characterized by mental retardation, speech impairment, gait ataxia and an inappropriate laughing and excitability. Microcephaly and seizures are common. It is caused by the loss of maternally imprinted contribution in the 15q11.2-q13 region. The evident existence of genotype-phenotype correlation suggests four phenotypic groups: patients with deletions, UPD (uniparental disomy) or ID (imprinting defect), UBE3A mutations and unknown aetiology. Patients with large chromosomal deletions and those with unknown aetiology are more severely affected than those with UPD or ID. Patients with UBE3A mutations fit in the middle of severity compared to the other groups.

Case report: We describe a 10.5-years-old Croatian girl with AS. Karyotype and FISH with D15S10 and SNRPN probes were normal, but parent specific DNA methylation analysis and DNA polymorphism testing for uniparenatal disomy showed UPD with both alleles FES/FPS of her father. She had normal weight and height, head circumference around 75. percentile, mental retardation with speech impairment and happy disposition with bursts of laughter. She had specific EEG pattern with.2-3 Hz large amplitude slow wave paroxysms, but no seizures at all.

Conclusion: The normal growth parameters, lacks of seizures or gait ataxia do not exclude the possibility of AS in child with mental retardation and happy disposition. EEG should be done in every child with cryptogenic psychomotor retardation because the characteristic EEG pattern appear early and is specific to the AS and one should expect the UPD or ID mechanism in the child with mild clinical spectrum of AS.

P0114. Croatian family with X-linked non-specific mental retardation due to interstitional deletion of Xq23 region

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Background: More than 100 X-linked mental retardation (MR) syndromes have been described with half of the genes mapped to specific regions of the X chromosome. MRX23 gene was mapped between DXS1220 and DXS424, at Xq23-24 in one family with affected male members presented with non-specific MR and verbal disability. We report three generations of single family with cytogenetically visible deletion of Xq23 region.**Case report**: The 36-year-old pregnant woman was referred to genetic counselling because of MR and autism in her family. Her parents were second-degree cousins. Three brothers were severely mentally retarded; one showing autistic behaviour. The Fragile X syndrome was excluded. The patient and her mother showed the same karyotype: 46,X,delX(q 23),t(1;12)(p36.2;q24.31). The same interstitial deletion was found in both brothers. An unbalanced translocation resulting in partial trisomy 1p and monosomy 12q was found in autistic brother. The cytogenetic analysis of amniotic fluid cells revealed an affected male fetus with interstitional deletion Xq23 and balanced translocation 1p;12q. The pregnancy was terminated.**Conclusion**: Our family represents the further evidence that certain regions of the X chromosome were enriched for genes responsible for MR. We propose that Xq23 region should be considered for precise cytogenetic and molecular diagnosis in such cases. The involvement of 1p and 12q region in the peculiar phenotype of one brother, showing also elements of autism, indicate the need for further analysis. The molecular analysis will be performed in order to get precise information about breakpoint sites and chromosomal segments involved.

P0115. Autopsy larynx findings of a patient with Down syndrome (DS, case report)

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Careful autopsy studies of larynx tissues are rarely done for DS. We adduce some findings of post-mortem examination of a girl with DS.

Proband was born after the fourth pregnancy by spontaneous vaginal delivery at term to a 44-yr-old female. Her parents had three healthy elder children. Proband had birth weight of 2600g (10centile)and length of 47cm (3centile). At 10th day of life diagnosis of DS was confirmed (karyotype 47,XX+21). She had multiple congenital malformations including open Bottallo's duct, atrial septum defect of the muscular part of ventricular septum, kidney dysplasia. The child 11 mn aged was dead following bilateral virus-bacterial abscess-forming pneumonia with fibrous-suppurative pleurisy. Autopsy findings of larynx are the following: there were tissue dysplasia of fibrous membrane with irregular thickness and adipose tissue inclusion, redundancy and irregular distribution of blood and lymphatic vessels of capillary type, wide zone of filamentous cartilage with solitary capillary vessels, solitary capillary vessels in hyaloid cartilage; there were irregular distribution of larynx glandulae with lobular and exodic duct malformations, often the ducts were separately disposed.

P0116. Expression of the features of Treacher Collins syndrome in members of two unrelated families

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Mandibulofacial dysostosis (TCSy) is an autosomal dominant disease which affects craniofacial structures. The most frequent symptoms in TCSy are bilateral symmetric anomalies of facial structures which originate from the first and second branchial arches. This includes antimongoloid slant of the eyelids, hypoplasia of the maxillar region, retromicrognathia, deformed ears and deafness. Although some authors claimed heterogenity of the syndrome, most of the cases represent fresh mutation of the TCOF1 gene, over 100 mutations have been described till now. The gene maps to 5q32-33, and it encodes nucleolar phosphoprotein treacle, which haploinsufficiency produces the signs of the syndrome.

Two unrelated families with 12 affected members of TCSy have been evaluated. Anthropometric evaluation have been done in all affected members in three generations, and in one family photographs of some members of the forth generation have been evaluated. The measurement of the facial features have been done according to the published data (Kolar, Arvystas), as well as audiological examination. Since the main features of the TCSy were very variable within family members, they were classified into three groups: mild, moderate, severe. In both families, the most affected members were probands, and the carrier parent was the mother. The most constant feature was antimongoloid slanting of the eyes and malformed ears. Anthropometric analysis of the narrowness of the midface and ear length differs significantly from normal.

When molecular analysis is not available, minor physical characteristics of the parents have to be carefully evaluated for proper genetic counseling.

P0117. Laterality defects - clinical study of 23 patients

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Situs inversus and situs ambiguous (heterotaxia) are terms used for complete or partial reversal of the normal position of lateralized viscera. They could be isolated or syndromic. Many genetic factors could be involved.

We present a clinical study of 23 patients with abnormal position of internal viscera in order to appreciate the frequency of different associated features, to underline their importance for the diagnosis and also to illustrate some particular cases.

Our study revealed higher frequency for males (60.9%) and total type of situs inversus (52.2%). Survival rate was high (78.3%). All the deceased cases had severe forms that associate heterotaxia with complex heart defects and other anomalies. The most frequent anomalies associated are: heart defects (69.6%, generally complex), renal (26.1%) and spleen defects (21.7%). The different types of heart anomalies will be analyzed separately. Syndromic forms were diagnosed as Ivemark syndrome, X-linked laterality sequence, Kartagener syndrome, poly/asplenia- corpus callosum agenesis- caudal deficiency, but also Marden Walker syndrome and Kabuki make up syndrome were identified. In order to optimize the management of syndromic forms, we have designed a protocol for the diagnosis and follow up of these patients. A comprehensive review of the literature referring to situs inversus, heterotaxia and the genetic factors involved will be presented.

In conclusion, the study presents our experience in the diagnosis of patients with abnormal position of internal viscera showing statistical data, particular cases, a management protocol and a comprehensive literature review.

P0118. Microcephaly with chorioretinal dysplasia (Alzial-Dufier syndrome): report of three patients and literature review

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We report on microcephaly and chorioretinal dysplasia, in 3 unrelated patients (aged of 3, 5, and 18 years). Microcephaly was present at birth for the two oldest patients (-2 SD and -3.5 SD), and has now reached respectively from youngest to oldest -3.5 SD, -5 SD and -4.5 SD. Developmental milestones were achieved appropriately, but the two oldest patients presented with mild mental retardation and speech delay (global IQ of 78 at 10 years old for the eldest). Clinical examination revealed bilateral clinodactyly of fifth fingers (2/3), persistent nystagmus (1/3), atrial septal defect (1/3), and supernumerary nipple (1/3). Ophthalmologic examination showed a chorioretinal dysplasia with punched-out lesions (3/3). Cerebral MRI showed peri-ventricular cysts located on the temporal lobes (1/3). Three distinct conditions with microcephaly and abnormal ophthalmologic findings have been delineated in the literature namely an autosomal dominant form (MIM 156590), an autosomal recessive form (MIM 251270), and an autosomal dominant variant associated with lymphoedema (MIM 152950). The mode of inheritance and the nosology of these syndromes are still questionable. Limwongse et al. described a family with features encompassing both the autosomal dominant variant of microcephaly with chorioretinal dysplasia and the autosomal form with lymphoedema. According to these data and to our reported cases, we therefore suggest that these 3 conditions may represent the same entity with a broad phenotypic variability. Finally, we emphasize the importance of ophthalmologic examination in patients presenting with pre- and/or post-natal microcephaly, as well as for both parents, even if the head circumferences are normal.

P0119. Familial X-linked cardiomyopathy

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Dilated cardiomyopathy is characterized by an increase in ventricular size and impairment of ventricular function. Familial dilated cardiomyopathy is usually considered to be a rare and distinct disorder. Between idiopathic forms, rare cases are with strong familial aggregation, early presentation in childhood and X-linked transmission.

Methods. We investigated a family with 8 children, using family history and physical examination, electrocardiography, echocardiography.

Results: Among the family members 4 children were affected (3 males, 1 female) The mean age at presentation was 8 ± 3 months with signs of severe heart failure. Two (2) children died after about 5 ± 2,5 month, at 23 month, through refractory heart failure, respectively at 15 month through sudden death. The third male had an embolic cerebral vascular accident, as a complication of dilated

cardiomyopathy, and has a poor prognosis.
The female showed a particular evolution, as she had severe heart failure as an infant, and is now completely recovered and symptom-free 7 years after. The rest of family members were examined, but none showed any clinical or echocardiographic criteria of dilated cardiomyopathy.
Conclusions: We consider that the presented cases belong to the rare form of familial X-linked cardiomyopathy, where the affected gene is the dystrophin encoding gene, that give us the explanation why the female recovered, while the males had a poor prognosis.

P0120. Association between minor congenital anomalies and learning disabilities

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Minor congenital anomalies do not have medical or cosmetic importance, but detection of more than three such anomalies may reveal the prenatal origin of a disorder. The aim of the present study was to establish an association between the presence of minor congenital anomalies and learning disabilities, attention deficit and hyperactivity. 219 patients aged between 7 and 18 years were examined for the presence of minor congenital anomalies and compared to a control group. The frequencies of these anomalies were not significantly different in the two groups. The mean values of minor congenital anomalies per child were significantly different: 2.5 in children with learning disabilities, 4 in those with learning disabilities and attention deficit and hyperactivity, 1.05 in the control group. As a positive correlation could be established, it can be concluded that the presence of minor congenital anomalies especially more than three, may predict the future onset of these disorders.

P0121. New clinical features of 3C syndrome

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Aim: to present a new case of 3C (or Ritscher-Schinzel) syndrome, a rare autosomal recessive syndrome characterized by craniofacial, cerebellar, and cardiac anomalies. Various other malformations are associated with 3C syndrome.
Case Report: A male infant, 2nd child of phenotypically healthy parents, was born after a normal delivery. At birth, body weight, length and head circumference was normal, but a general hypotonicity was recorded. Physical examination revealed a characteristic facial appearance with prominent forehead and occiput, hypertelorism, down-slanting palpebral fissures, low-set ears, depressed nasal bridge, and micrognathia. Skeletal anomalies included: camptodactyly of the thumb and the index finger of the right upper limb; bilaterally partial syndactyly of the 2nd and 3rd toes; hypoplasia of the 3rd toe bilaterally. Hypospadias and bilateral nonpalpable testes (intra-abdominally testes by sonogram). X-rays of the skull: dilatation of the sagittal suture and numerous Wormian bones in the position of the anterior fontanelle. Chest x-ray: a supernumerary right 13th rib. Eye examination: posterior embryotoxon (left). Echocardiogram revealed Fallot tetralogy. CT of the head: Dandy-Walker malformation with posterior fossa cyst, enlargement of cisterna magna, and cerebellar vemis hypoplasia. Chromosomes were normal.
Discussion: The presence of the testes in the abdomen, the posterior embryotoxon, and the Wormian bones of the skull are associated with 3C syndrome, for first time in the literature. These new clinical features further expand the phenotypic variability of 3C syndrome, and may be used in the differential diagnosis from several similar clinical entities.

P0122. Clinical heterogeneity in familial syndactyly

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Syndactyly is a congenital anomaly in human beings characterized by two or more fused fingers or toes.
This study presents three brothers with anomalies of the digits, in the context of a family with four children, the first one being an unaffected girl. Family history revealed that parents are healthy, unrelated individuals. There were no other cases among their relatives. All of these boys had synostosys of the distal phalanx (two bilateral and one unilateral), syndactylism of the third and fourth digits, bilateral clino-camptodactilly. Syndactyly occurs as a unique anomaly or as a part of a plurimalformative syndrome. Genetic and clinical heterogeneity, reduced penetrance and variable expresivity, as well as different possible etiologies (genetic, multifactorial or epigenetic) must be taken into consideration for the genetic counseling.

P0123. Zygosity Testing: Why Twins Or Their Parents Want To Know Zygosity

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At the University of Alberta Hospital between 1989 and 2003 there were 397 requests for zygosity testing by DNA analysis from parents of twins (361) or adult twins (36). Median age of testing was 3 years, range 0 days to 73 years. Tissues examined for DNA polymorphisms included placenta (65), blood samples (312), cord blood (5) and buccal swabs (15). Of all twins tested, 93% were monozygotic (MZ). In the 0-17 year-old age group, there were equal numbers of males and females but there was a predominance of females (83%) among the adults tested. Chorionicity was available in 147 cases. 89% of dichorionic (DC) twins were MZ, while 7% of histological monochorionic (MC) twins were dizygotic (DZ). Reasons for requesting zygosity were given in 184 cases. In the 0-17 year age group, 'need to know' and 'curiosity' accounted for 50% of requests. Health concerns accounted for 32% of requests from adult twins and 17% from children. These included discordance for anomalies or disease, stillbirth or neonatal death of one twin, IVF twins, family planning, recurrence of twins and future transplantation. Among the adult twins, four were discordant for cancer and two for renal disease. Other reasons included misinformation about zygosity; most commonly parents of DC twins were told their twins were DZ, and other twins in the family. Parents of twins or adult twins may be uncertain of zygosity from appearance alone and knowing zygosity for certain may be reassuring.

P0124. Myotonic Dystrophy In Yakutia

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Myotonic dystrophy (MD) is one of the widespread hereditary diseases in Yakutia (Korotov M.N., Kuzmina Z.M., 2000, Nogovitsina A.N., 2001). In this investigation we consulted 31 Yakut families with 74 patients (43 female, 31 male) affected by MD in the medical-genetic consulting clinic of the Republican Hospital -National Center of Medicine, Yakutsk. 37(50%) of the cases were marked as an adult form, 27 (36%) of the cases referred to a classical youth form, 8 cases (11%) were marked as an early child's form and 2 (3%) have congenital form of MD. Since 2002 a DNA-diagnostics of MD has been introduced by applying PCR and electrophoresis (to determine the number of CTG-repeats) in the laboratory of the medical-genetic consulting clinic in the Republican Hospital I -National Center of Medicine, Yakutsk. There were 217 diagnostic investigations on symptomatic patients (74 abnormal), 143 there healthy relatives (86 normal, 57 abnormal) and 5 prenatal diagnosis (2 normal, 3 abnormal). In 86 cases MD was excluded for the clinically healthy relatives (heterozygosity was detected for 2 normal alleles), an expansion size of CTG-repeats to be further determined in 74 patients and the rest 57 relatives. MD is a disease of importance at the national level.

P0125. Two new missense mutations in *RUNX2* cause a mild phenotype of Cleidocranial Dysplasia (CCD)

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Cleidocranial Dysplasia (CCD) is an autosomal dominantly inherited skeletal disorder, characterised by clavicular hypoplasia, large fontanelles with delayed closure, dental anomalies and short stature. CCD is caused by mutations of *RUNX2* located on chromosome 6p21 which encodes a protein that is part of the bone morphogenetic protein signalling cascade.
In this report we present two unrelated CCD families with novel missense mutations in *RUNX2* and relatively mild clinical phenotypes. The first family presented because of bilateral hip dysplasia in their 6-month-old daughter. Examination of the father revealed shortening of the clavicles and a funnel chest. Supernumerary teeth had been extracted at an earlier age. The body height of the father was not decreased (1,81 m), but his head circumference was enlarged. Molecular analysis of *RUNX2* revealed a proline- to leucine substitution in exon 2 (P127L).
In the adult patient of the second family the persistence of deciduous teeth and impaction of permanent teeth was the only clinically relevant symptom. Aplasia of the frontal sinus was demonstrated radiologically. Radiographs of the thorax and hand excluded hypoplasia of the clavicles and other skeletal symptoms in the index patient. His body height was normal, the head circumference enlarged.
In this patient a glutamine- to histidine substitution (Q209H) in exon 4 was identified.
Both of the above mutations are located in the highly conserved, DNA-binding RUNX-domain of the protein. Although both missense mutations led clinically to different phenotypes, the mild expression of CCD with absence of short stature was common to both patients.

P0126. Inherited thrombophilias and cytogenetics findings in patients with repeated fetal loss

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Reproductive genetics is a part of reproductive medicine. As a part of the preventive genetic examination in patients with repeated fetal loss we karyotype both partners and check for the Factor V Leiden mutation G1691A , the Factor II prothrombin mutation G20210A and the thermolabile variant ot the methylenetetrahydrofolate reductase gene in the woman.
Inherited thrombophilias are the leading cause of maternal thromboembolism and are associated with increased risk of certain adverse pregnancy outcomes. These effects imply an association between the factor V-Leiden mutation and late first-, second-, and third-trimester fetal loss.
There are conflicting data on the link between hyperhomocysteinemia (caused by hetero- or homozygosity for the C667T MTHFR thermolabile mutant) and recurrent spontaneus abortions.
The frequency of Factor V-Leiden in the Czech population is about 6%, of G20210A Prothrombin about 2.3%, of heterozygotes for C677T MTHFR about 38.2% and about 10% for homozygosity for C677T MTHFR.
In our group we followed 150 couples with repeated spontaneus abortions. We found an increased number of carriers of congenital chromosomal aberations in men and women in this group. The number of heterozygotes for C677T MTHFR was 47 % and homozygotes 6,7%. In women with three or more fetal losses we found 13.3 % carriers of factor V-Leiden.
We recommend to test for inherited thrombophilias in women with two and more repeated spontaneous abortions, a hematological examination and pregnancy monitoring in women -who are carriers of inherited thrombophilias.We test inherited thrombophilias in partners to detect the risk of homozygosity in the offspring.

P0127. *MECP2* related disorders in male patients with mental retardation and neurological symptoms

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Since Rett syndrome (RTT, MIM *312750) is known to be caused by mutations in the *MECP2* (methyl-CpG-binding protein 2) gene, mutations have been reported also in male patients. Among them is a group of males with non-mosaic and often familial *MECP2* mutations unknown to occur in females. They show a broad spectrum of neurodevelopmental disorders, most often mental retardation (MR) and divers neurological signs.
We tested a group of 70 males with an unexplained combination of MR and unselected neurological symptoms for mutations in *MECP2* by sequencing the coding region. All patients had undergone a careful clinical evaluation, cytogenetic analysis and most of them additional investigations. Patients with dysmorphism or with indications for an environmental cause of their MR were not included. 7 sequence changes were identified: a *de novo* missense mutation P225L (674C>T) in a male with RTT variant (for details see Eur J Paed Neurol 2003;07:5-12), an unclassified variant (1214C>T) and 5 non-pathogenic variants.
The validation of hitherto unreported nucleotide changes has to be done carefully and is hampered by e.g. inaccessibility of male family members and the rare occurrence of some variants. Also several males previously published to have *MECP2* related disorders were shown to have a non-pathogenic variant by further studies. In this clinically highly selected group, a low prevalence of pathogenic *MECP2* mutations has been found so far. Nevertheless, the clinician should be aware of RTT variants in males and consider *MECP2* analysis in males with MR associated with a progressive neurological disorder.

P0128. Genomics of Male Infertility

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In the last few years considerable progress has been made towards understanding sperm physiology and the biology of gamete interaction to understand the pathophysiology of male infertility. Also with advent of Assisted Reproductive Technology (ART) knowledge about the various factors leading to spermatogenic impairment is one of the most important aspects of scientific research.
Y chromosome deletions lead to infertility. Three regions on Yq (AZFa, AZFb and AZFc)are critical for spermatogenesis. So far four groups from India have published their data on male infertility using molecular methods.
Idiopathic oligozoospermic and azoospermic cases were included in this study. Cytogenetic and semen analysis was done in each case. In cytogenetically normal cases microdeletion analysis was done according to guidelines as prescribed by European academy of andrology. Microdeletions spanning the AZF loci were found in a 9.63% cases and genotype, phenotype correlations exists. . In a large number of cases with AZFc microdeletion there is a population of germ cells with XO cell line and these cases are counseled prior to going in for ART about the risk of male offspring being born with sexual ambiguity, turner phenotype in addition to being infertile.
Deletion on Y chromosome make the Y chromsome more prone to secondary larger deletions resulting in worsening of testicular phenotype. Thus in a large number of idiopathic cases of male infertility there is a genetic basis and in the other normal cases need to further evaluated.

P0129. Mild Phenotypes in a Series of Opitz GBBB Syndrome Patients with MID1 Mutations

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Opitz syndrome (OS) is a congenital midline malformation syndrome characterized by hypertelorism, hypospadias, cleft lip/palate, laryngotracheoesophageal (LTE) abnormalities, imperforate anus, developmental delay and cardiac defects. The X-linked form (XLOS) is caused by mutations in the MID1 gene, which encodes a microtubule-associated RING finger protein. In this study, phenotypic manifestations of patients with and without MID1 mutations were compared to determine genotype-phenotype correlations. MID1 mutation analysis of 70 patients with clinically suspected OS revealed 14 different mutations in 25 of the subjects. These included 19 patients from 8 different pedigrees with familial XLOS, 4 sporadic cases, and 2 patients with XLOS of unclear inheritance. Ten novel mutations are reported here for the first time. The XLOS patients with MID1 mutations appeared to be less severely affected than previously reported, particularly in functionally significant domains such as LTE, anal, cardiac and neurologic abnormalities. Less functionally relevant minor anomalies were more prevalent in patients with MID1 mutations. Female XLOS carriers had much milder phenotypes compared to male MID1 mutation carriers, with the most ubiquitously penetrant trait being hypertelorism. Most of the anomalies found in patients of this study do not seem to correlate with the type of molecular MID1 gene defect, with the possible exception of LTE malformations. This study highlights the wide spectrum of severity and manifestations of OS, reflecting the genetic heterogeneity of the disease and complexity of the underlying mechanisms. It also shows that genetically confirmed XLOS patients may have a better prognosis and quality of life than previously thought.

P0130. Multiple café-au-lait spots and three different malignancies in two siblings of a consanguineous marriage between first cousins

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Multiple café-au-lait spots (CLS) are usually the first clinical sign of neurofibromatosis type 1 (NF1) in affected children. We report here on two siblings of unaffected consanguineous parents, who developed multiple CLS and early-onset malignancies. The girl of the family was diagnosed with medulloblastoma at the age of 7 years and with myelodysplatic syndrome (MDS) two years later. She died from an acute myeloid leukaemia (AML) into which the MDS has evolved. Her brother, who also presented with CLS, was diagnosed with a high grade glioblastoma at the age of 9 years and is currently in stable condition. *NF1* mutation analysis revealed no evidence for a constitutional truncating *NF1* mutation nor a *NF1* microdeletion in the boy. Neither did FISH and LOH analyses reveal evidence for loss of one *NF1* allele in the leukaemic cells of the girl. Early-onset tumours and signs of NF1 have been reported in so far nine children with inherited homozygous deficiency of one of the mismatch repair (MMR) genes. All children presented with CLS and developed either haematological malignancies or brain tumours or both. Six children were found in three HNPCC families, however, in two families there was no family history of colorectal cancers. Therefore, we suggest homozygous mismatch repair deficiency may be the genetic alteration causing cancer predisposition and NF1 symptoms also in the here presented siblings. We are currently performing mutation analysis in the mismatch repair genes, MLH1, MSH2, PMS2 and MSH6 and autozygosity mapping of loci associated with these genes.

P0131. Clinical genealogical and electrophysiological analysis of the hereditary motor -sensory neuropaties in Moldova and polymorphic short tandem repeats for diagnosis of the Charcot-Marie-Tooth 1 A duplication.

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The inherited neuropathies of the peripheral nervous system are clinically and genetically a heterogenous group of disorders. Molecular genetic studies have made major breakthroughs in unraveling the underlying gene defects, and DNA diagnosis can now be offered to a large number of families with distinct forms of hereditary peripheral neuropathies.We presents the results of clinical-genealogical and electrophysiological examination of patients with hereditary motor-sensory neuropathies, which were included in long-term registry of Moldova. Some genetics features of the disease have been revealed (intrafamilial polymorphism of different degree, effect of the ancestor in the dominant forms). Electrophysiological peculiarities of the separate clinical-genealogical variations of the pathology were found. According to publication date most CMT1A cases (>98%) are caused by a duplication of a 1.5 Mb region on the shot arm of chromosome 17 containing the PMP22 gene. We test 2 STRs located within the duplication (tetra- and pentanucleotide repeats) 54 DNA samples of unrelated CMT1 patients . The CMT1A duplication was determine in 24.07% cases (gene dosage for heterozygous samples- different fluorescent intensity and /or three alleles). The presence of two alleles (59.3%) was used to indicate that no duplication was present in samples. Homozygous samples 16.6% required a competitive gene dosage test. The PMP 22 duplication is not the most frequent abnormality in Moldavian's CMT1

P0132. Molecular study of the SMA patients in Iranian Spinal Muscular Atrophy families

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of anterior horn cells in the spinal cord leading to progressive muscular weakness and atrophy. The SMA-determining genes including SMN (survival motor neuron), the responsible gene in SMA phenotype expression, NAIP gene (neuronal apoptosis inhibitory protein) and BTF2P44 are located on chromosome 5q13 in two highly homologous copies (telomeric and centeromeric) within the SMA region.
In this study, the NAIP gene deletion was analyzed in 34 SMA families, with the consanguinity rate of 65% (22/34), in which exon 7 of the SMNt gene deletion was already confirmed and was deleted in 79% of affected individuals. We found 63% (12/19) NAIP gene deletion in SMAs (83% SMA-I, 28% SMA-II and III), 6.6 %(2/30) of carrier parents for SMNt. The NAIP gene deletion in SMA-I was higher than other SMA-types.

P0133. Mental retardation and blepharophimosis in seven cases: overlapping features between Young-Simpson and Ohdo syndrome

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The combination of blepharophimosis and mental retardation can be part of many genetic conditions. There are two distinct syndromes in which the latter are associated with multiple congenital anomalies and characteristic facial features. However, although there are overlapping features between these two conditions, hypothyroidism and post-axial polydactyly seem to be specific findings of Young-Simpson syndrome, whereas congenital heart defect, hearing impairment and dental anomalies have only been described in Ohdo syndrome. Here we report 6 patients with overlapping features of theses two conditions.

Our seven cases shared common findings such as facial features including blepharophimosis, a wide nasal bridge, dysplastic ears and a micrognathia; mental retardation; bilateral talipes and/or camptodactyly. Four patients presented with raised TSH and optic atrophy in addition to the initial findings. Five cases presented with long thumbs, and one case presented with unilateral post-axial polydactyly. Two patients presented with hypoplastic teeth and three with overlapping toes. Two cases presented with hearing impairment. Our seven patients have got overlapping features of both syndromes and it is difficult to establish a definite diagnosis. Diagnosis is important for genetic counselling, as autosomal recessive inheritance has been proposed in Young-Simpson syndrome. The main clinical characteristics of Young-Simpson/Ohdo syndromes will be discussed, and the cases will be compared with those previously reported.

P0134. Oto-Facio-Cervical syndrome: Identification of a large constitutional deletion of the 8q13 BOR syndrome region

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Oto-facio-cervical (OFC) syndrome is a rare autosomal dominant condition that has been described in four unrelated patients. It overlaps clinically with the Branchio-Oto-Renal syndrome (BOR) syndrome. In addition to the manifestations found in BOR syndrome patients it is characterized by facial abnormalities, hypoplasia of the cervical muscles (pronounced sloping shoulders), mental retardation, and short stature. In 2001, Rickard et al. presented evidence that the OFC syndrome is a contiguous gene deletion syndrome involving the EYA1 gene that is mutated in BOR syndrome.
We describe a male patient with hypoplastic kidneys who underwent transplantation at age 11. He presented with conductive hearing loss, small dysmorphic ears, a preauricular pit, lateral cervical fistulas, facial anomalies, a long neck with slopping shoulders and a pseudopterygium formed by the trapezius muscle. In addition, he had hexadactyly of the right hand and the right foot. By FISH-analyses we detected a deletion in the BOR syndrome region that was smaller than the deletion seen by Rickard et al. (2001). Our results will help to narrow down the critical region of the OFC syndrome.
Literature: Rickard et al., Hum Genet (2001) 108 :398-403

P0135. Evaluation of clinical findings in four patients with Williams syndrome

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Williams syndrome is characterized with dysmorphic face, mental retardation, characteristic behavior profile, idiopathic hypercalcemia, as well as supravalvular aortic stenosis (SVAS). This syndrome results from a microdeletion in chromosome 7 (7q11.23) including the elastin gene named ELN, which synthesize the elastin protein, an important protein in connective tissue.
Clinical and laboratory findings of four patients referred for facial dysmorphism with congenital heart disease, diagnosed as Williams syndrome and confirmed by FISH analysis, were evaluated. FISH technique was applied after the features of the patients were evaluated using the scoring system recommended by American Pediatrics Academy in 2001.
Two of the patients were male, and two were female, and all had congenital heart disease. One patient had VSD and PDA, one had mild pulmonary stenosis, one subaortic VSD and pulmonary stenosis, and one had pulmonary stenosis and secundum ASD. Besides, there were major dysmorphic clinical findings.
It was emphasized that Williams syndrome, which is considered to be a rare genetic disease, is actually more frequently seen genetic disease of childhood, when clinical findings are carefully analyzed and diagnostic laboratory tests are used. The findings were discussed in the light of the literature.

P0136. Skewed X-inactivation is associated with recurrent spontaneous abortion

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Recurrent spontaneous abortion (RSA) is defined as three or more consecutive losses at 20 weeks or less of gestation. A significant proportion of the unexplained cases of RSA may be caused by a genetic mutation or chromosomal abnormality. The role of nonrandom X chromosome inactivation (XCI) has been questioned in the pathogenesis of RSA in recent studies but conflicting results obtained (Sangha et. al., *Am. J. Hum. Genet.* 65, 913-917, 1999, Sullivan et al. *Obstet Gynecol.* 101, 1236, 2003). We therefore investigated the XCI patterns in peripheral blood DNA obtained from 64 RSA patients and 160 age matched controls. Pregnancy history, age, karyotype information and disease information was collected from all subjects. The methylation status of a highly polymorphic CAG repeat in the androgen-receptor (*HUMARA*) gene was determined by use of methylation-sensitive restriction enzyme *Hpa*II and PCR. The PCR products, both before and after digestion, were separated on 8% sequencing gel, were dried, and were autoradiographed on Medicalfilm CP-BU (Agfa). Densitometric analysis of the alleles was performed using the Multi-Analyst software version 1.1 (Bio-Rad Laboratories). Skewed X-inactivation (>80 percent skewing) was observed in 13 of the 53 informative patients (24.5 percent), and 10 of the 124 informative controls (8 percent) (P=0.005; χ^2 test). More importantly, extremely skewed XCI, defined as >90% inactivation of one allele, was present in nine (16.9 percent) patients, and in only three controls (P=0.001; χ^2 test). These results support the interpretation that disturbances in X-inactivation mosaicism may be involved in the pathogenesis of RSA.

P0137. Genotype-phenotype correlation in Portuguese families with hereditary deafness associated to GJB2 mutations

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Most cases of moderate-to-profound congenital inherited deafness are caused by mutations in the GJB2 gene. Over one hundred Cx26 mutations have been identified so far. Consequently, molecular screening for GJB2 mutations has become an important tool in the diagnosis of hereditary prelingual deafness.
GJB2 is considered to have allelic heterogeneity since different mutations cause different phenotypes and a given mutation can cause different levels of hearing loss, even within the same family. No clear genotype-phenotype correlation has been established to date, which would have important implications for genetic counseling and prognostic clinical information. In a recent collaborative study involving a large number of pacients segregating two GJB2 mutations, a first evidence of correlation was obtained.
In the present study we have analysed portuguese families with biallelic mutations in GJB2, in order to assess a possible genotype-phenotype correlation. This analysis enabled us to conclude that the Cx26 mutations identified in portuguese pacients cause hearing impairment of any degree of severity, with inter- and intra-familial variability, as previously reported for other populations. Most Individuals homozygotic for the 35delG mutation presented severe to profound hearing impairment, but in a few cases this genotype was also associated to moderate impairment. A slight deficit at high frequencies was observed in several 35delG carriers.
These data represent a contribution for the establishment of more precise genotype-phenotype correlations regarding GJB2 mutations.

P0138. Desmin storage restrictive cardiomyopathy with atrio-ventricular block is associated with desmin gene defects.

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Background. Desmin storage restrictive cardiomyopathy (RCM) is causally linked to defects of desmin and alpha-B crystallin (*CRYAB*) genes. 13 mutations have been identified to date in the desmin gene (chr. 2q35), 12 in patients with desmin storage myopathy, and one in a familial dilated cardiomyopahty in which the myocardium has not been investigated.

Methods and Results. We report four desmin gene mutations, three novel and one known, in four unrelated probands diagnosed with RCM plus atrioventricular block treated with pacemaker implantation. Of the four probands none had clinically overt skeletal muscle involvement at the time of diagnosis. Their heart disease was diagnosed at the mean age of 30±11 years. Molecular genetic analysis identified 4 mutations of the desmin gene: R16C, R406W, T453I, delGTATACCTTG splice site junction (exon 3). Screening of the Alpha-B Crystallin gene gave negative results. The disease was familial autosomal dominant in two cases, autosomal recessive in one and associated with a *de novo* mutation in one. Overall, 17 members of the four families underwent clinical and instrumental screening as well as molecular genetic analysis. Of the 17 members of the 4 families, 8 all affected carried the desmin gene defect.

Conclusion. Desmin storage disease involving the heart causes a typical RCM plus atrioventricular block. Although two genes have been reported as associated with the disease, the desmin gene is the most likely candidate. The typical phenotype constitutes the easy cardiological marker for addressing these patients to EMB and molecular genetic analysis of the desmin gene.

P0139. Revisiting metatropic dysplasia: report on 19 new patients and review of the literature

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Metatropic dysplasia (MD) is a rare chondrodysplasia characterised by short limbs with limitation and enlargement of joints and usually severe kyphoscoliosis. Radiological features include severe platyspondyly, severe metaphyseal enlargement and shortening of long bones. The term “metatropos” means changing patterns in greek and emphasizes the evolutive changes in the body proportions of patients with MD. The classification proposed by Beck et al, based on the radiological features, divide three different types: a lethal autosomal recessive form, an autosomal recessive non lethal form and a non lethal autosomal dominant form with less severe X-rays manifestations and a better clinical outcome.

Here, we report on clinical, radiological and histological findings of 19 new MD patients (5 lethal or terminated cases, and 14 living patients) and compared them with 67 patients previously reported in the literature.

Beside usual diagnostic criteria, additional radiological features have been observed ie hypoplasia of the anterior body of first cervical vertebrae, irregular and squared calcaneum bone and precocious calcification of hyoid and cricoid bones. Moreover, the long term follow up of one patient from birth to 30 years shows radiological features overlapping two different types (ie the non lethal autosomal recessive and the dominant form).

Finally, among the 76 patients, the observation of 2 families only with recurrence and two cases with vertical transmission is suggestive of a “unique” autosomal dominant mode of inheritance which is confirmed by the use of the statistical proband method (Chi2 = 8.31).

P0140. MYBPC3-linked hypertrophic cardiomyopahty is not a “benign” disease.

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Background. Preliminary data on genotype-phenotype correlations in patients diagnosed with hypertrophic cardiomyopathy (HCM) due to myosin-binding protein C (MYBPC3) gene mutations, indicate that MYBPC3 gene defects are associated with late onset and good prognosis-phenotype. The aim of the present study was to record events (death and cardiac transplantation) and follow-up data [congestive heart failure (CHF), implantable-cardioverter defibrillator (ICD), worsening of the New York Heart Association (NYHA) functional class] of 35 patients with HCM due to MYBPC3 gene defects.

Methods and Results. 116 consecutive patients (71 males, mean age 41±16 years), diagnosed with HCM, underwent clinical evaluation and molecular genetic analysis. This latter identified 35 (30%) mutations of MYBPC3 gene, in 35 unrelated patients: 26 novel mutations were novel (73%), 7 known (27%). After 63±14 months of follow-up we recorded 3 deaths (2 sudden cardiac deaths and one due to congestive heart failure). Eight patients developed cardiac dilatation and dysfunction: 2 of them underwent heart transplantation (HTx), 2 ICD implantation for ventricular arrhythmias, 4 worsened the NYHA functional class from I to II; one patient developed restrictive pattern and was implanted with ICD for ventricular arrhythmias. The remaining 23 patients (65%) are in stable clinical conditions, 10 in NYHA class I and 13 in NYHA class II.

Conclusions. Of 35 unrelated probands with HCM causally linked to MYBPC3 gene defects, 12 (34.3%) developed major events or worsened after 63±14 months from diagnosis. The disease is therefore not a benign phenotype as preliminarily suggested.

P0141. Genetic mapping in families with hereditary syndactylies

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The combination of molecular and genetic studies with experimental manipulation of developing limb buds has significantly advanced our understanding of the signal centers, signalling molecules and their complex molecular interactions, co-ordinating limb bud outgrowth and patterning. One of the approaches to understand limb development is the study of human subjects showing various limb deformities. We intend to identify gene(s) causing syndactylies. These genes might influence anterior-posterior patterning or the separation of individual finger rays.

Four families with hereditary non-sydnromic syndactylies were ascertained from various parts of Pakistan. The phenotypic study of the affected subjects revealed that one of these families segregated type I syndactyly, two families syndactyly type II, all of them showing autosomal dominant mode of inheritance. The fourth family, depicting an autosomal recessive inheritance was proved to be a unique type, hitherto unreported.

Since there were three chromosomal locations known for type I, II and III syndactyly (2q34-q36, 2q31, 6q22-q23, respectively), we performed linkage analysis using highly polymorphic microsatellite markers from these regions in three of the families to look for cosegregation of the phenotype with any of these loci. Our findings indicated that the phenotype in these families was not linked to any of these loci and there was genetic heterogeneity for syndactyly type I and II, beyond the known loci. Also it confirmed that our family with recessive syndactyly was a unique variant with an unknown novel locus.

Ultimately we intend to identify and characterize the candidate gene(s) involved in the disease phenotype in our families.

P0142. Williams-Beuren-Syndrome: Determination of deletion-breakpoint on chromosome 7q11.23 using quantitative Real-Time-PCR

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The Williams-Beuren-Syndrome (WBS) a rare (1/20000-50000) genetic disorder is associated with a 1.5-2Mb hemizygous deletion on chromosome 7q11.23. WBS-patients display vascular stenosis, weakness of connective-tissue, dysmorphic face, short stature and mental retardation.
At least twenty-four genes have been identified in the deletion-region in WBS-patients flanking by large low-copy-repeat sequences (> 320Kb).
Haploinsuffiency of the Elastin-gene (ELN) and most probably of the LIMK1-gene has been shown to be causally involved in the pathogenesis of WBS. Isolated hemizygosity of ELN leads to a supravalvular aortic stenosis, the hemizygosity of the LIMk1 gene is responsible for the impaired visual-spatial-constructive cognition in WBS-patients. The flanking genes of ELN are assumed to cause other feature of WBS such as mental retardation, hypercalcemia and connective tissue abnormalities.
The precise identification of deletion-breakpoints and determination of the correct size of the deletions is not feasible by using the FISH- and/or microsatellites analysis.
For determination of the deletion-sizes we developed a quantitative PCR-approach (qPCR) using SybrGreen® dye and reactions were analysed on an ABI-7900HT (TaqMan). Our assay screens 2.2Mb of the WBS-region in 50Kb intervals.
This methodology has been tested in DNA samples of ten patients with a known deletion of the WBS-region and was able to detect the deletion and the approximate size in all cases.
We will investigate further DNA samples including patients with a strong clinical suspect of WBS but without apparent deletion. We will report on the detection efficiency and sensitivity of this new system and we will perform a genotype/phenotype-correlation.

P0143. Cutis laxa arising from an autosomal dominant splice mutation in exon 24 of the elastin gene (ELN) in a three months old boy and his father with highly variable phenotype

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Congenital cutis laxa is a rare, genetically heterogeneous connective tissue disorder. The systemic involvement of elastic tissue is characterized by high clinical variability. The typical aspect is marked skin laxity lacking elastic recoil which is accompanied nearly always by rarefication, loss, fragmentation or severe disorganisation of dermal elastic fibres. In three cases of autosomal dominant cutis laxa, causal frameshift mutations within the gene coding the structural protein Elastin, a main component of elastic fibres, could be identified. Here we report a three month old boy with cutis laxa and his clinically unaffected father. Mutation analysis in the Elastin gene was carried out by direct sequencing all coding exons in genomic DNA. A cytosine to thymine substitution was identified at the last position of exon 24. To further characterize the implication of the mutation, total RNA was extracted from cultured fibroblasts. RT-PCR and subsequent sequencing of a fragment encompassing the critical region revealed an in frame deletion of exon 24, resulting in a stable protein lacking amino acids 526-540. This is consistent with former observations that suggest that the phenotype of cutis laxa is the result of a dominant negative effect compared to SVAS caused by loss of function mutations in the ELN gene. Interestingly, the mutation was also present in the father who had no signs of cutis laxa, suggesting that the mutation is associated with a high phenotypic variability. Nevertheless, herewith we report the fourth family with autosomal dominant cutis laxa, caused by a mutation in the Elastin gene.

P0144. Italian experience of DM1 molecular testing on 710 two-generations families.

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Myotonic dystrophy type 1 (DM1; OMIM #160900) is an autosomal-dominant genetic disorder with multisystemic clinical features associated with a CTG expansion in 3' untranslated region of the dystrophia myotonica protein kinase gene (DMPK) on chromosome 19q13.3. DM1 genotypes can be divided into E1 (50-200 CTG), E2 (200-1000 CTG) and E3 (>1000 CTG) classes. We report on a 6 years experience in providing symptomatic and prenatal molecular diagnostic services for DM1 over the Italian territory on 710 two-generations families. Analysis of the intergenerational differences in repeat length has revealed an allelic dimension increase in 64.1% of analysed families, in each case concordant with the DM1 clinical anticipation characteristics. Contraction events were observed in 6.4% of cases, while the repeat is stable in the remaining 29.5% of germ line transmissions. We have then subdivided our data on the basis of DM1 mutation parental origin. We found that the CTG repeat length increase in 180 of male and in 275 of female transmission respectively. We observed more frequently an E1-E2 passage (male =115 and female =105) and an E2-E3 passage (male =25 and female =130), more rarely E1-E3 change (male =40 and female =40). In conclusion : 1) the DM1 mutation is more stable through generations than expected; 2) the CTG repeat tends preferentially expand maintaining a well defined genotype-phenotype correlation; 3) there are no significant differences in DM1 mutation stability between paternal and maternal transmission.
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P0145. Holt-Oram syndrome: a french national collaborative study in a series of 31 families

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Holt-Oram syndrome (HOS) is the most frequent “heart-hand” syndrome. Autosomal dominant inheritance is the rule in this condition which expression can be highly variable. Clinically, HOS is characterised by the association of cardiac malformations and pre-axial anomalies of the upper limbs.
HOS has been ascribed to TBX5 mutations since 1997 in 30% of the cases, highlighting the genetic heterogeneity of this entity.
A national French collaboration led to the clinical analysis of 31 families since 2002. We have established precise clinical diagnostic criteria allowing to classify the families in 3 distinct categories: typical HOS (19), atypical HOS (9), and highly improbable HOS (3). Molecular biology was performed in 25 families, and is still pending in 6.
Amongst the “atypical” (8) and the “highly improbable” (3) cases, no TBX5 mutations were identified. In the 14 “typical” families, 11 mutations have been identified, including 6 which were not previously reported (3 nonsense, 2 missense and 1 frameshift).
Although we can not yet establish genotype-phenotype correlation in our cohort, we confirm the genetic heterogeneity of HOS and the interest of precise clinical delineation before molecular analysis.
We will search for TBX5 deletion for the “typical” cases with negative mutation screening, and we will look for other candidate genes in the “atypical” cases.

P0146. X-Linked Mental Retardation in the Iranian Population

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With an incidence of 1.5 to 2.5 percent, mental retardation (MR) is one of the biggest unsolved problems in Medical Genetics. Severe forms affect approximately 0.5 percent of the general population, and most of these have genetic causes. An excess of affected males is a consistent finding in all published studies. Based on these observations, it has been estimated that 25 percent of the severe genetic forms of mental retardation may be due to gene defects on the human X chromosome. Recent molecular studies in families with syndromic and non-syndromic X-linked MR have already revealed mutations in close to 50 different genes. However, it has become apparent that in general, mutations in these genes are very rare. In Western populations ,the Fragile X syndrome, does not account for more than 2% to 2.5 % of all cases.
In Iran with its high proportion of consanguineous marriages, mental retardation is a particularly important problem. As a prelude to large-scale, systematic research into X-chromosomal and autosomal forms of MR, we have implemented molecular Fragile X diagnosis and tested 387 probands from 249 families with possible X-linked inheritance patterns. 134 patients had a full mutation , 32 probands carried a premutation , and 221 did not have recognizable mutations in the FMR1 gene. These results may indicate that in Iran, Fra(X) is more common than elsewhere. In the non-Fra(X) families that are large enough, linkage studies and mutation screening are being performed to rule out defects of other known genes for XLMR.

P0147. Williams Breuren syndrome associated with caudal regression syndrome and coagulopathy

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Here we report on the clinical and molecular genetic findings in a patient with the quite unusual association of Williams Beuren syndrome (WBS) with caudal regression syndrome (CRS) and a rare variant of a coagulopathy (combined deficiency of factor XI and factor XII). Molecular cytogenetic analysis confirmed a de novo deletion of the WBS-critical region including the elastin gene and the LIM-Kinase 1 gene in 7q11.23. The precise extent of the deletion, which in this case can not be recognized in banded chromosome preparations has still to be determined. At least one form of CRS has also been mapped to the long arm of chromosome 7 but the locus for the autosomal dominantly inherited Currarino syndrome at 7q36 is much more distally located. An independent deletion of the HLXB9 gene which could be responsible for the formation of CRS was ruled out by FISH analysis, as was the presence of a paracentric inversion at the WBS critical region in her mother. Unfortunately her father was not available for analysis yet. There are various types of coagulopathies, however the combination of a deficiency of factor XI and XII is not well documented nor is there an established linkage to 7q. It remains to be investigated by sequencing of the HLXB9 gene and detailed mapping of chromosomal breakpoints flanking the WBS deletion to rule out a coincidence or to show that there is indeed a causal relationship between an unusually large albeit still submicroscopic deletion with that phenotype.

P0148. Fibrillin 1 (FBN1) genotype versus Ghent criteria in 65 unrelated probands with suspected Marfan Syndrome.

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MFS (MIM#154700) is an autosomal dominant disorder (1:5000) of the connective tissue primarily involving the cardiovascular, ocular and skeletal systems (Ghent nosology). Its diagnosis relies on Ghent criteria among which positive family history and FBN1 mutations are major ones. FBN1 is a very large gene (15q21: 230 Kb, 65 exons, 2871 amino acids). To date, 559 mutations have been identified. The study aimed at assessing whether FBN1 mutations found in MFS patients match Ghent criteria and clinical phenotypes.
Clinical series: 40 unrelated probands (11<18 and 29>18 years) and relatives. FBN1 screening: DHPLC analysis of the 65 exons and sequencing of heteroduplex amplicons. C677T MTHFR was analysed with RFLP. Controls: one hundred chromosomes (50 unrelated healthy individuals).
Of the 40 FBN1 mutations, 24 were novel: Y20C; C224R; Q348X; R439G; Q454P; R516X; ex15-del15bp (nt2018-2031) and del8bp (2050-2057); ex19-delT (nt2467); G1058D; ex26-Ins10bp (nt3361); ex29:Y1219C, delC(nt3763); ex 30-Ins5b (nt3900-3904); ex41-IVS40-1A-G; R1790X; C1876Y; C1900Y; ex49-del2bp (nt6185-6186); ex54-ins5bp (nt6785); A2383Y; C2535W; Y2629C; ex63-delT (nt8032).
Of the above 40 patients, 19(47.5%) fulfilled the Ghent criteria: 3/19 aged <18 years. 18(45%) met two criteria plus FBN1 mutation: 7/18 aged <18 years. 3(7,5%) met one Ghent criterion and carried the FBN1 mutation: 1/3 aged <18 years. Three pairs of unrelated families shared identical mutations but phenotypes within families showed different severity. C677T As expected, Ghent nosology met the age of the patients, and FBN1 genotype constituted a major diagnostic contributor to diagnosis in patients <18 years.No correlation was found between FBN1 genotype, C677T variation and severity of the phenotype.

P0149. Otoferline : The trap of the neonatal hearing loss screening with the evoked otoacoustic emissions

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Hearing loss is the most frequent of the sensory deficits. One child in 1000 at birth presents with a severe or a profound deafness, while another one in 1000 becomes deaf before adulthood. Recent discoveries in human genetics have indicated that 60-80% of cases of congenital deafness are of genetic origin in developed countries . The vast majority, about 85%, of non syndromic sensorineural hearing loss (NSSNHL) show autosomal recessive transmission. Since 1994, more than 70 loci for NSSNHL have been identified and 35 different genes cloned. One of them, GJB2, encoding connexin 26, is involved in the most frequent form of NSSNHL: DFNB1.
The OTOF gene encodes a protein, Otoferlin, wich is localised in the inner hair cells . This molecule could be involved in the fusion of the synaptic vesicules in the cochlear neuro-ephitelium. The mutations in OTOF are responsible for a congenital profound non syndromic deafness : DFNB9. The characteristic of DBNB9 is the persistance of transient evoked otoacoustic emissions, leading to the false negative result in case of hearing loss screening with this technique or leading to a diagnosis of auditory neuropathy.
We will present the phenotype and the genotype study of two independent patients presenting OTOF biallélique mutations. The molecular diagnostic has permited to localise the defect in the cochlear and to propose cochlear implant to the two families.

P0150. Further delineation of the clinical phenotype associated with mutations in *OPHN1* gene: a clinically recognizable syndrome

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A mutation in the *OPHN*1 gene, encoding Oligophrenin, has been reported for the first time in a family of non-specific XLMR. Since then , it has been demonstrated that mutations in *OHPN1* were associated with syndromic mental retardation with cerebellar hypoplasia in two families and a sporadic case (Philip et al, 2003; Bergmann et al, 2003). These findings were confirmed by the reappraisal of the first

family, demonstrating similar MRI findings. We report here a new family with the same phenotype. We made a review of the literature in order to propose a better definition of the clinical phenotype. Neuroradiological findings were identical in all male patients: cerbellar hypoplasia predominating on the lower vermis, cortical atrophy and variable ventricular dilatation requiring ventriculo-peritoneal shunting in two cases. Mental retardation was constant, usually moderate (IQ around 50), predominating on language. In all cases tested, verbal IQ was significantly lower than performance IQ, and all had rather conserved visuo-spatial abilities. There was no associated ataxia, except in one family. Strabismus was present in three families, epilepsy in two. In addition, we demonstrated that all male patients exhibit a characteristic facial appearance. Heterozygous females exhibit mild mental retardation and slight facial dysmorphism. All five mutations are non-sens mutations or small out-of-frame deletions. No reccurent mutation or hot-spot was observed, demonstrating that a complete mutation screening of the gene is mandatory in suspected cases.

P0151. A Case of Robinow Syndrome

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Robinow Syndrome which was first described in 1965 is characterised by fetal facies, hemivertebrae and genital hypoplasia. It can be inherited in both autosomal dominant and autosomal recessive fashion. Phenotypic features are more severe in the recessively inherited form. Up to date about 100 cases have been reported

A 16-month old boy was hospitalised because of his short starture and genital anomaly. There is no consanguinity between parents and there is no similar case in the family. On physical examination his weight was 9 kg (3-10th centile), height 71.5 cm (below 3rd centile), head circumference 46.5 cm (10th centile). He had all of the cardinal features of the syndrome including fetal face, hypertelorism, wide palpebral fissures, anteverted nostrils, triangular mouth, clinodactyly, brachymelia, umbilical hernia, hypospadias, and mesomelic dwarfism Laboratory tests revealed low IGF-1 , IGF-1 BP-3 , and low growth hormone levels. On X-ray examination multiple fusion defects in lumbar vertebrae and butterfly vertebrae, and mesomelic shortening of the extremities were observed. Cranial MR showed that the sella was normal.

This case is presented because of its rarity and the presence of low GH, IGF-1, IGF-1 BP 3 levels without empty sella.

P0152. Hidden 45, X/47, XXX/47, XX, del(Y)(p?)/46, XX mosaicism associated with True Hermaphroditism (TH)

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True hermaphroditism (TH) is an unusual form of sex reversal, characterized by unequivocal testicular and ovarian elements in the same individual. Approximately 60% TH have a 46, XX karyotype, 33% are mosaics with a second cell line containing a Y chromosome, while the remaining 7% are 46, XY. The Sex determining gene SRY, is present in only 10% of true hermaphrodites with a 46,XX karyotype, therefore in the remaining 90%, mutations at unknown X-linked or autosomal sex determining loci have been proposed as factors responsible for testicular. TH presents considerable genetic heterogeneity with several molecular anomalies leading to the dual gonadal development in a single subject. In 1998 gonadal SRY mosaicism was proposed in 3 sporadic 46,XX TH SRY- negative in blood leukocytes but positive in gonadal tissue and in 2002 the presence of hidden mosaicism for Y-derived sequences was confirmed as al mechanism to explain TH etiology in some cases. Here we report a new TH case in which a hidden mosaicism for the Y and X chromosome was detected by PCR and FISH in peripheral blood and gonadal tissue, supporting the fact that hidden mosaicism is associated with TH and that molecular analyses of gonadal tissue should be done in all 46,XX cases.

P0153. Two new patients with Cross (oculocerebral syndrome with hypopigmentation) syndrome.

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Syndromes with hypopigmentation of skin and hair include: i) isolated oculocutaneous albinism syndromes, ii) Chediak-Higashi, Griscelli, Vici and Hermansky-Pudlak syndromes, all characterized by hypopigmentation and immunodeficiency or haematological features, and iii) other heterogeneous disorders namely Tietz syndrome, ermine phenotype (or BADS syndrome), osteoporosis and oculocutaneous hypopigmentation syndrome, Preus syndrome and Cross syndrome (CS).

Here, we report on two brothers born from Portuguese first cousin parents. They both share the same phenotypic presentation namely hypomelanosis involving the skin, hair and eye, mild premature aging, mild mental retardation, sensorineural deafness, severe myopia, hypogonadism with testis ectopia, enamel anomaly and moderate postnatal short stature. Additional features are observed namely cerebellar atrophy (1/2), complete 2-3 cutaneous syndactyly of the toes (1/2) and glomerulopathy (1/2). Bone x-rays, high resolution blood chromosomes and immunological studies are normal. Ophthalmologic exam reveals fundal appearance of albinism.

The generalised hypopigmentation of skin and hair and the likely autosomal recessive mode of inheritance are suggestive of CS but the observation of mild mental retardation, myopia (despite the fundal appearance of albinism) and the absence of ataxia may also suggest the diagnosis of Preus syndrome. These diagnoses will be discussed in the light of the review of the literature.

P0154. 25 years of experience in the treatment of the cleft lip and palate

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Background Cleft lip and palate or orofacial cleft is one of the most common congenital malformations.

The average incidence is around 1 in every 1000 live births with variable birth prevalence based on geographic origins, the highest rates commonly beeing found in Asian populations.

Method The present study analizes a number of 290 medical records of pacients affected by the disease ,treated at the Childrens Hospital ,Louis Turcanu' ,Department of Pediatric Surgery in Timisoara,Romania during 1.01.1979-31.12.2003.

Results During this period of time we observed a more frequent incidence of cases in our male patients and also a higher number of complex (both lip and palate) cases mainly affecting the area around the cities of Petrosani and Anina in Romania.Ten percent of all the studied cases had a familiar history of the disease . The Millard gingivoperiosteoplasty technique was by far the most used followed by the Steffensen, Mesurier procedures . The Veau and Tennison techniques were used in 4 cases. Following these surgical procedures the results were very good in 80 % of cases ,good in 9 % of cases ,fair in 7 % and poor results were noted in 4 % of cases. Redone surgery after the correction procedure was necessary in 1 % of patients. Speech disorders affected mainly the patients with cleft palate in 12% of cases .

Conclusions Although the disease is known for a long time no genetic therapy made it's presence noted in solving the problem of cleft palate and lip.

P0155. Bartsocas- Papas syndrome: prenatal diagnosis and autopsy findings

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The Lethal Multiple Pterygium syndromes (LMPS) are a group of

genetically heterogenous conditions characterized by ptergyium, hypertelorism, hydrops fetalis and cystic hygroma. The Bartsocas-Papas syndrome (BPS) consists of pterygium, ankyloblepharon, a hypoplastic, malformed nose, a small jaw, oral synechia and limb abnormalities. We report a case of a fetus with many findings consistent with BPS, however, with some characteristic features missing; and some present usually reported in other LMPS.
The parents were non-consanguineous and the pregnancy was terminated at 28 weeks gestation for multiple abnormalities detected on fetal ultrasound. These include severe symmetrical IUGR, moderate oedema, omphalocele and rocker bottom feet. The autopsy showed pterygia of the popliteal, antecubital fossa and the axillae. There was partial syndactyly on both upper and lower limbs. Ankyloblepharon were absent. There were clefts of the palate and mandible. Synechia from the tongue to the mandibular alveolar ridges, and mucosal overgrowths involving the upper and lower pre-molar areas. The genitalia were hypoplastic and the karyotype was 46, XY.
The case highlights the continued difficulty of differentiating between the various syndromes associated with multiple pterygium. The shear number of pterygium, and the involvement of the axilla are more typical of LMPS. However, the oral synechia and genital hypoplasia fit well with BPS. Other concistent features of BPS, such as ankyloblepharon and a markedly malformed nose were absent. This case questions again the classification of the multiple pterygium syndromes. More definitive conclusions will be obtained with the finding of the different causative genes.

P0156. Several Silver-Russell-syndrome-like phenotype features in a female patient with a subtelomeric deletion 10q

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Here we present a 23 year old female patient, who is just 116 cm tall, with 23 kg. She shows a lateral asymmetry and several anomalies and dysmorphic features. She was born at term as the second child of a non consanguineous Austrian couple. Length was 41 cm and weight 1191 g. She did not drive well and was early diagnosed of having a Guerin-Stern syndrome because of some minor arthrogrypotic limb contractures mainly of her shorter right leg and her hands. Physical examination reveals that her right leg is 4.5 cm shorter and also her right arm is reduced in length. Her femora are disproportional short, she has missing ribs and a severe scoliosis. Chromosome analysis with 4 weeks showed a normal karyotype. In the first year a ventricular septal defect was corrected. Increased CPK and fructose-aldolase was repeatedly found but EMG was normal. Her psychomotor development is grossly retarded. She was not able to walk before 5 years and started to speak with 4 years. Additional anomalies were recognized like a double kidney left and just 2 month ago a vaginal atresia was diagnosed requiring surgical treatment with removal of her uterus. On this occasion a human genetic examination was performed and detailed chromosomal analysis including subtelomeric FISH revealed a cryptic 10q deletion. Although several of the reported symptoms are in good accordance with previously reported patients this particular case shows some additional features that will be presented together with a precise molecular characterization of the deletion.

P0157. Frequency of Alpha-1-Antitrypsin Pi*Z and Pi*S alleles in children with chronic liver disease in Brazil

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Alpha-1-Antitrypsin Deficiency (A1ATD), an autosomal recessive disorder characterized by reduced serum A1AT, is the most common genetic cause of liver disease in infants and children. More than 90 variants of the A1AT protein have now been identified. The most common alleles are Pi*S and Pi*Z which are characterized by serum A1AT concentration between 50 - 60% and 10 - 15% of normal, respectively. The aim of this study was to determine the frequency of the alleles Pi*S and Pi*Z in children with chronic liver disease.
DNA samples of 156 chronic liver disease children with ages between 1 month and 15 years were analyzed by polymerase chain reaction followed by digestion with *Taq*I and *Xmn*I enzymes.
Twelve children were homozygous and another 12 were heterozygous for the Pi*Z allele (7.7%). There were 3 children Pi*SZ and 2 were Pi*SS. Fourteen patients were heterozygous for the Pi*S allele. The frequency found for the Pi*Z allele was 12.5% and the Pi*S was 6.7%. The frequency of Pi*Z alleles in a previous study in a sample of our population was 0.5% and the Pi*S allele was 7.0%. The high frequency of Pi*Z in this population demonstrates the usefulness of A1ATD molecular testing in children with chronic liver disease.

P0158. Artificial neuronal networks as an aid to diagnose multiple congenital malformation syndromes

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Computer programs which can be used as an aid to diagnose multiple congenital malformation syndromes have been used for many years. These programs are based either on algorithms, which define a diagnosis by a set of phenotypic components all having the same weight or on algorithms based on a concept of Bayesian statistics. A new approach for this field are artificial neuronal networks (ANNs). A commercially available shell, suitable for building up feedforeward ANNs trained by using backpropagation of errors. The data of 234 patients representing individual examples of 21 different malformation syndromes were used. The number of symptoms or symptom combinations for the description of cases were limited to 28, 55 and 78 in different series of tests. After the export of the patients data to the shell the data of about 2/3 of the patients were used for the training of the ANN. The remaining 1/3 of patients data-sets were used to test the diagnostic capacities of the different ANNs. The most efficient yielded a diagnosis in more than 95 % of all tests. Correct diagnoses without any concurrent differential diagnoses were generated in 26 %; adding all tests producing the correct diagnosis among other diagnoses amounted to 74 %. The application of ANNs in the diagnostic process of malformation syndromes is efficient - one shortcoming is, that different sets of training data produce models with different generalization accuracies. The significance of an ANN is to a great extent influenced by chance and the experience of the developer.

P0159. A case of fetal hydantoin syndome having hemangioma associated with hemiatrophy at the left side of the body

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Fetal Hydantoin Syndrome occurs due to in utero exposure to hydantoin and only 10% of the infants born with adverse affects. The teratologic affects of hydantoin depend on the genotype of fetuses and include four major categories of problems: craniofacial dysmorphology, prenatal onset of growth deficiency, central nervous system dysfunction with IQ less than 75, increased risk for major malformations such as congenital heart disease, cleft lip/palate, microcephaly, and major genitourinary, eye, and limb defects.
A two years old girl was referred to us for a wine-colored pigmentation at the left side of her body and the shortness of her left extremities. She was born to an epileptic mother who was given phenytoin throughout thepregnancy. She was born spontaneously at term and she weighed 2900 g at birth.
Physical examination of the case showed broad depressed nasal bridge, short nose with bowed upper lip, broad alveolar ridge, high palate, short neck, low-set hairline, widely spaced nipples, umbilical hernia, hypoplasia of distal phalanges, hypoplastic fingertips, digit anomalies with small nails at her feet, large skin hemangioma and hemiatrophy at the left side of her body. The case has been diagnosed as Fetal Hydantoin Syndrome with an additional findings of hemangioma and hemiatrophy.
The association of fetal hydantoin syndrome and hemangioma with hemiatrophy has been described for the first time in this case

P0160. Genetic Characterization of Spondylothoracic Dysplasia

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Spondylothoracic dysplasia is an autosomal recessive disorder with high prevelence in Puerto Rican population. Typical findings include segmentation and formation defects of the spine. Short and rigid neck, protuberant abdomen and inguinal hernias are frequent findings as well. We have followed 32 patient for over 8 years allowing us to determine the natural history, phenotype and genotype charcaterization of the disease. Sequential pulmonary function tests, establishments of antropomorphic measurements and comparison of height and thoracic circumferences, have allow us to determine the minimal measurements required for survival. Genetic mapping to chromosome 2q31 has been establish and further characterization and reduction of the critical region to 2cM has been achieved. These has allowed us to establish the genetic and phenotypic differences among syndromes with similar phenotypes.

P0161. The clinical phenotype in 34 patients with supernumerary marker chromosomes containing the Prader-Willi-Angelman critical region

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We studied 20 females and 14 males aged 2-57 years with supernumerary marker chromosomes (SMC) containing one or more copies of the Prader-Willi-Angelman critical region (PWACR). Two were normally functioning individuals who were mosaic for the SMC; the rest were ascertained via learning and/or behavioural difficulties. Of the latter, 3 who were mosaic were among the more mildly affected. All the SMC were maternal in origin and all except two were de novo. One familial case was unusual in containing only a single copy of the PWACR, associated with a correspondingly milder phenotype, the other had a clinically normal mosaic mother.
Affected individuals typically showed severe learning disability, plus problems of social communication and behaviour which satisfied formal criteria for an autism spectrum disorder. About 80% suffered from seizures of various types, 60% had a history of hypotonia and early motor delay, 40% gave a history of strabismus, and 40% showed incoordination, usually mild. In contrast, the prevalence of major and minor congenital anomalies was low.
Although the breakpoints involved in the formation of the SMC varied considerably (see Roberts et al, Am J Hum Genet 2003, 73:1061-1072), no correlation was detected between specific clinical features and the inclusion of specific loci, only between overall severity and the number of additional copies of the PWACR as a whole. Where a normal cell line was detected, the clinical picture was less severe, and there was a possible association between a more severe outcome and the intensity of seizures.

P0162. The Psychosocial Aspects of Skeletal Dysplasia and the Impact of Molecular Genetic diagnosis

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The aim of this study was to assess the psycho-social problems associated with undiagnosed skeletal dysplasias and the impact of a precise molecular diagnosis. Patient recruitment was through the 11 of the UK regional genetic services. The genetic testing was provided by the regional molecular genetics laboratory (RMGL) in Manchester. Mutation screening was undertaken for patients with differing skeletal dysplasias. 129 diagnostic patient DNA samples had been received for mutation screening analysis (experimental group), with a separate 57 patients composing of the control group (no mutation analysis). Psychosocial assessment was undertaken at two interval points; 3 months prior to the disclosure of the test result and 3 months post test result. A similar course was employed for the control subjects. The psychological evaluation was performed using a series of semi-structured schedules and validated psychological measures. At this preliminary stage of analysis the results show that 40.8% of the patients had their diagnoses confirmed, 45 % had their previous diagnoses disputed and 14.2 % of the patients were given an unclassified diagnosis. After the second interview stage those patients who have received a genetic test were much more clear in their minds about their diagnosis and what it meant (38%), than those individuals who have not received a genetic test (15%). The findings suggest that the psycho-social and practical benefits of a molecular diagnosis for patients and their families is important.

P0163. Glucocerebrosidase gene: identification and characterization of four novel point mutations, a recombinant allele and a non-pseudogene-derived complex allele causing Gaucher disease type I

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Gaucher disease (GD) is the most frequent lysosomal glycolipid storage disorder due to an autosomal recessive deficiency of acid β-glucosidase (GBA) characterised by the accumulation of glucocerebroside. Three GD clinical variants have been identified based in the absence (Type 1) or presence of primary CNS involvement (Type 2, acute neuropathic and Type 3, subacute neuropathic). The GBA gene (~7 kb) is located on chromosome 1 and contains 11 exons. A highly homologous 5 kb-pseudogene is located 16 kb downstream from the active gene.
In this work we characterised six novel genetic alterations in GBA gene causing GD type 1: four missense changes (D24N, I119S, P182L, V191E), a recombinant allele and a non-pseudogene-derived complex allele (G421D+H451R). All six mutant alleles were present as compound heterozygotes in association with N370S, the most common mutation in GD1. The mutant alleles, except the recombinant one, were expressed *in vitro* in COS-1 cells and analysed by enzyme activity, protein processing and intracellular localization.
The novel allele due to the recombination between the gene and the pseudogene was identified by the amplification of a fragment from intron 4 to intron 7 in which an anomalous product of 1743bp was obtained, cloned and sequenced. The1743bp fragment contained the gene sequence up to intron 5: the first nucleotide change A>G corresponding to those of the pseudogne was detected at nucleotide position 4179 of GBA gene. Recombination extended up to exon 7 where the mistmatch C>T at genomic nucleotide position 5152 corresponding to the pseudogene was not present.

P0164. The importance of alternative communication for Cri-du-Chat syndrome: a case study

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This work intends to show that early stimulation and utilization of alternative comunication can change significantly the prognosis of Cri-du-Chat syndrome. This syndrome presents as major characteristics problems with cognitive and motor development. Speech acquisition is also a major problem for children with this syndrome. To achieve the objective, it will be made a case study, based on a brazilian child - Clarice Sant'Anna - who is now 4 years and six months. Since she was born, she has been stimulated with physical therapy and speech therapy. Since her one year and eight months, Clarice attains regular scholling. When she was two years, she has begun sign language as alternative means to communication. With that, she has acquired ability to communicate with her parents. In addition, it has provided a significant reduction in attention's deficit, has ended steriotyped moviments, increased her internal language and, finally, has developed her desire to speak. Another type of alternative communication has been begun to turn possible Clarice to speak with her classmates and school teachers: it has been used for that the Picture Symbols Communication - PCS - photographs and products labels. As a result of these therapies, Clarice is now able to speak a few words very clearly and has much

more capacity to keep concentrated in school activities. It is also worth mentioning that she can walk since she was three years.

P0165. Prader Willi Syndrome to mat UPD 15, associated with large deletion of long arm of one Chromosome 15, including the entire FBN1 gene

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In a newborn with clinical and anamnestic aspects evocative of PWS, we applied the due protocol of laboratory studies.
The constitutional karyotype was normal 46,XY, and the FISH analysis didn't reveal submicroscopic deletion of the PWS/AS critical region. The methylation test confirmed the clinical diagnosis of PWS. By using a battery of DNA polymorphic markers, we were able to identify a maternal heterodisomy for the entire chromosome 15: during this research, quite unexpected was the identification of a large "de novo" deletion of the long arm of one of the two maternal chromosomes 15.
This large deletion includes the entire FBN1 gene, as well demonstrated by using intra and extra gene markers.
Some reports of the literature describe very rare large deletions of FBN1 gene; no deletion of the entire Fibrillin gene was until now described.
The child, now 2-years old doesn't show clinical patterns of Marfan syndrome. The ultrastructural study of skin and connective tissues, didn't reveal important pathological aspects . Studies on the collagen and Fibrillin protein are now in progress.

P0166. Genetic analysis of familial stuttering

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Stuttering is a speech disorder, which begins in childhood between the ages of 3-6 and is characterized by involuntary syllable repetitions, sound prolongations or interruptions (audible and silent). It is a painful symptom and is considered as society's hidden disability. Stuttering greatly interferes with a child's emotional and psychological development and also affects their daily social or occupational functioning. The incidence of stuttering among the general population is about 0.7-1.0% with a higher 4-5:1 ratio of male to females affected. Family, twin, and adoption studies strongly suggest a genetic predisposition/susceptibility to stuttering, but no genes have yet been identified. Associations between childhood and/or adult stuttering and brain disorders, such as Down syndrome, Parkinson's disease, Tourette syndrome and depressive illness, have been reported. The gene responsible for stuttering has been suggested on 18p and proximal 18q (Am J Med Genet. 124A: 133-5, 2004). We have studied a large six generation Indian pedigree with an autosomal dominant stuttering (OMIM 184450). The pedigree consists of 260 individuals including 95 affecteds (58 males/ 37 females). The onset is ranged from 3-7 years. The age distribution of affecteds is 8-70 years. Severity of the phenotype was quite variable among the affecteds and skipping of a generation was also observed. Karyotype analysis of thirty-five sporadic affecteds with stuttering showed chromosomal anomaly in one individual. Linkage studies with markers closely linked to chromosome 18 will both confirm allelism to this locus and may reduce the genetic interval encompassing the stuttering gene or provide evidence for genetic heterogeneity.

P0167. Neonatal Marfan Syndrome: two cases with some unusual clinical findings

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We report two cases with unusual clinical findings. Case1: female born at 37 weeks gestation with loose redundant facial skin, deep-set eyes, aracnodactyly, joint contractures and the hepatic veins connecting to the right atrium, previously unreported in nMFS. A right congenital diaphragmatic hernia was diagnosed prenatally at 28 weeks gestation. The baby developed collapse of much of the left lung and lower segment of lower right lobe. A missense mutation c. 3202T>G on exon 25 of *FBN-1* gene was detected. This mutation occurs in calcium biding epidermal growth factor like domain 11. Fibroblasts studies are in process. Case 2: male infant born at term with dolicomacrocephaly, deep set eyes, loose redundant skin over head and neck, pectus carinatum, marked arachnodactyly, joint contractures, mitral valve regurgitation and dilatation of aortic and pulmonary roots. The brain MRI revealed interdigitation of the interhemispheric fissure suggesting a partial absence of the falx and a cerebellum displaced upwards through a dehiscent incisura, also seen at first time in a nMFS patient. Neurological examination was normal. At 3 months, the patient died secondary to severe mitral valve insufficiency. A novel G>A transition mutation was identified and it is predicted to affect the invariant +1 position of the splice donor site in intron 30 leading to skipping of exon 30 of the *FBN-1* gene. By the fibroblasts studies of case 2, we can speculate that skipping of exon 30 does not affect the initial process of fibrillogenesis and elastogenesis, but rather contributes to a decreased durability of elastic fibers, possibly linked to increased expression of elastolytic proteinase(s).

P0168. Genetic basis of assessment of carrier status and risk estimation in spinal muscular atrophy (SMA)

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Spinal muscular atrophy is one of the most common neuromuscular disorders of early childhood with an incidence of about 1:10,000. Due to the high incidence and fatal outcome in a high percentage of affected children, genetic testing is of utmost importance not only for confirmation of the diagnosis in affected children but also for defining the carrier status in relatives of SMA patients and their spouses. Approximately 95% of clinically typical SMA patients lack both copies of the SMN1 gene. The interpretation of results of heterozygosity tests in SMA is, however, complicated by different factors: 1.) About 1.7% of SMA disease alleles show other small intragenic SMN1 mutations. 2.) About 1.8% of patients show a spontaneous mutation. 3.) About 3.3% of SMA chromosomes in the total population have two SMN1 copies. Despite the growing knowledge about the molecular basis of SMA and the increasing use of heterozygosity testing risk calculation is often wrong. The main reasons are: 1.) Calculation of risks without Bayesian theorem. 2) Not taking Haldane's mutation-selection equilibrium (1935) into account and 1.) The uncritical use of incidence data. Results of precise risk calculation often differs to a great extent from figures given in clinical practice or the majority of publications about this subject. On the basis of the mentioned criteria risk assessment of common situations will be given, underlining the necessity of knowledge of formal genetics in the molecular era of genetics.

P0169. A new phenotype associating sensory and motor neuropathy with a predominant proprioceptive ataxia

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Sensory ataxia is a symptom almost associated with either a cerebellar syndrome in the context of spinocerebellar atrophy (SCA), or a superficial sensory deficit in the framework of Charcot-Marie-Tooth disease (CMT) and hereditary sensory neuropathy type I (HSN I).
Sensory ataxia is rarely a major symptom of hereditary neuropathies with AD transmission.
We describe a French family in which segregated an AD sensory and motor neuropathy, characterized by late onset of symptoms and sensory ataxia. This family comprises 96 members including 26 affected individuals.

Genotyping of 40 microsatellites markers covering 8 candidate loci was performed on an ABI 3730 sequencer: CMT 1A, 1B, 2A & 2B, HSN 1A & 1B, sensory motor neuropathy with ataxia (SMNA/SCA18) and spinocerebellar ataxia 25 (SCA25).
14 examined patients had normal muscle strength except 9 cases where mild weakness in distal lower limbs was observed. In all cases, vibratory and pinprick sensations were decreased or abolished in distal limbs and deep tendon reflexes were absent or depressed. Electrophysiological examination showed mildly reduced motor and sensory nerve conduction velocities with severely decreased compound muscle action potential (CMAP) and sensory nerve action potential amplitudes. Peroneal nerve biopsy showed a severe loss of myelinated fibbers with clusters of regeneration and few onion bulb formations. These findings are consistent with a primary axonal degeneration with some demyelinating features. At the genetic level, the 8 candidate loci were excluded by haplotyping and linkage analysis.
This neuropathy corresponds to a new phenotype variant of CMT2. A genome-wide search is in progress to localise the responsible gene.

P0170. Pregnancy Loss and Prospective Medico-Genetic Consulting

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In the system of Medico - Genetic consulting of the families with Pregnancy Loss, cytogenetic examination is obligatory.
The aim of this work is to study the frequency of chromosome aberrations among families with Pregnancy loss according to data of Medico - Genetics consulting room of Kaluga Regional Hospital.
As a result of cytogenetic research in the selection of 252 patients (126 couples) chromosome aberrations were revealed in 16 patients.
Table 1 Cytogenetic aberrations revealed in families with Pregnancy Loss

Number of families	Aberration	women	men
1	46,XX,t(13;7)	+	-
1	46,XX,t(17;13)	+	-
1	45,XX,t(14;21)	+	-
4	46,XX,inv (9)	+	-
1	46,X inv(X)	+	-
1	46,XX/45,X	+	-
1	46,XX/47,XXX	+	-
1	46,XY,t(6;11)	-	
1	46,XY, inv (Y)	-	
1	46,XY,del (Y)	-	
1	46,XY,inv (9)	-	
1	47XYY	-	
1	47,XY + mar	-	

Dash - normal cariotype
According to data obtained total chromosome aberration in families examined made up 12,7%. Cariotype pathology among men - 4,76% correspondingly. Data obtained among women fully correspond the results of other research. Cariotyping among men showed higher figures of chromosome aberrations in comparison with the data of other authors. In our opinion it is connected with the expansion of existing indices of cytogenetic examination of the families with Pregnancy Loss, it is the presence of phenotype healthy children in families and miscarriages in anamnesis that didn‘t exclude the possibility of revealing balanced aberrations both among men women.
So, the indices used by us, are applicable both for retrospective examination of families with burdened obstetrical anamnesis and for prospective medico-genetic consulting of couples who are going to marry having relations with reproductive aberrations in their genealogy.

P0171. HVI and HVII mitochondrial DNA polymorphism in Turner syndrome: analysis of 28 mother-child pairs

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Molecular biological studies on Turner Syndrome (TS) during last years have contributed to clarify that the 70-80% of 45,X patients retain the maternal X chromosome. MtDNA is inherited maternally and the haplotype is used to study maternal lineage. Some evidences indicate that interaction of the sex chromosome and mtDNA might exist, therefore we have analyzed HVI and HVII segments of the non coding mtDNA control region in 25 mother-affected child pairs 45,X maternally inherited and 3 mother/patient pairs with Y chromosome mosaicism. Cytogenetic data were confirmed by X microsatellites and amelogenin analysis.
The HVI and HVII sequences were compared with normal Italian population data without identifying an unique haplotype for the TS patients but low frequency mutations were found . Mother-child haplotype patterns comparison showed differences in mtDNA sequence. At present the significance of this difference remains to be clarified.

P0172. Identification of the origin of a marker chromosome 7 in a child with dysmorphic signs and congenital heart defect

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We report on a case of additional marker chromosome mosaicism. Cytogenetic analysis was performed postnatally and revealed mos 47, XX + mar [26] / 46, XX [14]. The karyotypes of the parents were normal. The marker chromosome was identified by fluorescence in situ hybridization (FISH) and derived from chromosome 7. A marker chromosome derived from chromosome 7 has not been reported until now. A uniparental disomy 7 was excluded. The child revealed congenital heart defect and dysmorphic signs. The psychomotoric development of the little girl was retarded and followed up for two years.

P0173. Chromosome heteromorphisms in couples with reproductive failure

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In order to estimate the role played by chromosome heteromorphisms in reproductive failure an analysis was carried out on 128 couples with sterility, recurrent abortions or stillborn infants. The couples were selected in the Clinic of Gynecology “Bega” Timisoara - In Vitro Fertilization Department (2001-2003). Using the protocol with ethidium bromide (10µg/ml) and GTG and C banding techniques we were able to identify 31 couples (24.2%) with chromosome heteromorphisms. Among them one couple presented a different variant in both partners and in two couples one partner had two different variants (1qh+ and 9qh+). In 21 couples (67.6%) chromosome 9 showed an increased secondary constriction (15 couples) or a pericentric inversion (6 couples). 10 couples (32.3%) revealed an augmentation of the size of the h region of chromosome 1 (9 couples) and 16 (1 couple). The size of the hererochomatin variant was appreciated using 16p as a reference standard (small - 42.4%, intermediate - 27.3%, large - 12.1%). The highest variation in size has been recorded for the h region of chromosome 9. The pericentric inversion of chromosome 9 showed different pattern in size and location of the breakpoints.
The chromosome heteromorphism could be regarded as a risk factor in couples with reproductive failure.

P0174. Hirschsprung disease and retinitis pigmentosa in a boy with interstitial deletion of chromosome 13q22q31

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We described a 45 days-old boy with dysmorphic facies consisting

of round flat face with short and broad nose, prominent glabella, hypertelorism and blue, discolored eyes. His second toe, especially the distal phalanx was broad and bifid fingernail overlapping the first toe was noticed in the right foot. Chromosome 13 deletion was found at (13)(q22.1q31.3) region by cytogenetic analysis and FISH investigation. The patient was followed-up until he was 2.5 years old. Psychomotor development was delayed. Since the patient's deletion region was the Hircshsprung disease gene region, a rectal biopsy was performed at 10 months of age because of increasing complaints of constipation. Biopsy demonstrated aganglionic megacolon. He also had retinitis pigmentosa which not been described in 13q deletion syndromes.

P0175. Some cytogenetic aspects of primary amenorrhea

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Eight cases with cytogenetic abnormalities were found among 53 patients with primary amenorrhea.
The karyotypes of these cases were:
θ 46,XY in four cases with histologic confirmation of testicular feminisation syndrome for three of them
θ mosaicism with X monosomy:
-46,XX/45,X/47,XXX (82%/13%/5%) with Turner stigmata, 1 case
-45,X/46,XX (63%/37%) without Turner stigmata, 1 case
-45,XX,t(20;21)/45,X, 1 case involving autosomes
θ mosaicism without X monosomy:
-45,XX,-14,+18p/46,XX,+18pter (20%/80%), 1 case
Our cases are clear examples of the different mechanisms involved in primary amenorrhea.
If the role of X mosaicism is well established in primary amenorrhea, only few knowledge concerning autosomal involvement in the pathogenesis of sexual development were accumulated.

P0176. Gonadal mosaicism of partial trisomy 12p

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We report on the unusual case of a partial trisomy 12p mosaicism in a proband without any phenotypic abnormality. The index patient was a 16-year-old boy affected by mental retardation and craniofacial dysmorphy including macrocephaly, a short nose with anteverted nostrils and a broad protruding lower lip. G-banding and whole chromosome painting-FISH analysis in cultured lymphocytes revealed a derivative chromosome 12 with a partial duplication of the short arm: 46,XY,dup(12)(p12.1p13.3).ish dup(12)(wcp12+). Chromosome analyses in parental lymphocytes showed a normal male karyotype in the father. In contrast, chromosomal mosaicism was detected in the phenotypically normal mother with 12% cells exhibiting the same derivative chromosome 12 as detected in her son. In the mother, chimerism was excluded by the analysis of microsatellites. 10 years before, lymphocyte analysis in the mother had also revealed a trisomy 12p mosaicism with 11.5% abnormal cells. This demonstrates the high stability of the aberrant cell line. As a consequence of the chromosomal aberration in the son, it can be concluded that a gonadal mosaicism is present in the mother.

P0177. Genotype/phenotype discrepancies in 2 cases of Down Syndrome: a „de novo" trisomy 21 and a complex translocation of maternal origin

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Trisomy 21 or Down Syndrome (DS) is easily identified clinically. Since the discovery of the chromosomal region responsible for its main phenotypic characteristics (21q22.2) it is now possible to explain the rare cases of discrepancy genotype/phenotype of some patients.
Patient 1 was referred because of developmental delay, recurrent vomiting and weight loss. Although the cytogenetic investigation revealed a 47,XY,+21 karyotype, the classic phenotype of DS was absent. The parent's karyotypes were both normal. FISH analysis using a specific probe for the Down Syndrome Critical Region (DSCR) (locus D21S55, in 21q22.2) demonstrated the absence of this region in one of the three chromosomes 21, thus explaining the lack of the typical dysmorphic features. Final karyotype: 47,XY,+21.ish del(21)(q22.2q22.2)(wcp21+,D21S55-).
Patient 2 was referred because of simian crease, low-set ears and developmental delay. The karyotype was interpreted as a reciprocal translocation of maternal origin involving chromosomes 5 and 21, with an extra 21: 47,XY,t(5;21)(q12.3;q21.2)+21mat. Since the phenotype was not typical of trisomy 21, complementary FISH studies showed that chromosome 3 was also involved in the familial translocation - the mother had the DSCR translocated onto chromosome 3 (which was not inherited by the child). Therefore besides the 2 normal copies of chromosome 21 (with 2 signals for the DSCR), the child had partial 3p and 21q trisomies . Final karyotype: 47,XY,der(5),der(21),t(3;5;21)(p24.3;q13.1;q22.1)mat,+21.ish 21q22.2(D21S55x2)
Given the rarity of discrepant clinical and cytogenetic assessments of DS the authors emphasise the importance of good communication between clinicians and laboratories to allow precision and accuracy of diagnoses.

P0178. Two cases of de novo partial duplication of 1p: molecular cytogenetic characterization by high resolution multicolor-banding (MCB) FISH and clinical aspects

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Isolated partial duplications of short arm of the chromosome 1 are rare findings. We report on two unrelated patients with duplications within 1p, their karyotype abnormalities both initially were described by GTG-banding analysis as 46,XX,add(1p).
Multicolor banding (MCB) assay using the chromosome 1 probe set was applied to investigate the abnormal chromosomes. Thus, the origin of additional material of 1p was discovered and the precise breakpoints were characterized.
Proband 1 is a 6-years-old girl with mental and speech delay, congenital heart disease, multiple malformations. Isolated partial duplication was showed in her karyotype: 46,XX,add(1).ish dup(1)(pter→p36.3::p36.2→qter) de novo.
Proband 2 is a 10-years-old female with mental and speech delay, behavior difficulties and multiple anomalies. Inverted duplication was described as:
46,XX,add(1).ish dup(1)(pter→p36.2::p31.3→p36.2::p31.3→qter) de novo.
We demonstrated the high effectiveness of MCB approach for diagnostics of unclear interstitial rearrangements. MCB elucidates the orientation of duplicated segments and clarifies the mechanism of chromosomal aberration. Duplications described in two probands covered essential part of 1p and had a common breakpoint at 1p36.2 while the sizes and localization of duplicated segments were different. The clinical spectrum of pure partial trisomies 1p is discussed with regards to literature data cases and to size and origin of duplicated 1p segments.
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P0179. Acrofacial dysostosis-Nager typeand alobar holoprosencephaly in a case with a previously undefined chromosomal abnormality

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The present case was terminated after multiple anomalies noted on ultrasound at 17 weeks gestation. The phenotypic features were proptosis, anasia, facial cleft with complete cleft palate, atresia of the left external ear, short neck, absent thumb on the left hand, simian line on the left hand, clubfoot and postaxial polydactyly of the left foot. The autopsy findings were alobar holoprosencephaly, truncus arteriosus, lobe anomaly in the lung (single lobe on the right, two

lobes on the left), right renal agenesis, absence of uterus, fallopian tubes and both ovaries.
The chromosome analysis of skin fibroblasts showed 45,XX,der(5) t(5;22)(p14.3;q11),-22.
Acrofacial dysostoses (AFD) are a heterogenous group of disorders involving mandibulofacial dysostosis and various acral anomalies. This fetus had a lethal type of AFD. Among the various types of AFDs this case represents Nager-type AFD base on the facial dysostosis and preaxial limb defect. Genitourinary abnormalities have also been reported. However congenital heart defect is very uncommon and holoprosencephaly (HPE) has not defined previously. Both AFD and HPE have been separately described with deletions in different chromosomes. This is yet the first case clinically combining both entities with a previously undiagnosed cytogenetic abnormality.

P0180. A new subtelomeric 4p deletion

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The Wolf-Hirschhorn syndrome (WHS), is a wellknown syndrome whose critical region seems to be located at D4F26 locus at 4p16.3. The microdeletion of this critical region generates the clinical manifestation of the syndrome. Nevertheless, with the new molecular techniques, several cases with features resembling the WHS, had a 4p microdeletion which not involve the critical region of this syndrome but a proximal zone. This could suggests that D4F26 locus is not the only critical deletion site for clinical WHS.
We present a case with a subtelomeric deletion of the region D4S3360 in 4p, (localized at 73 kb from the telomere, Cytocell), and with no deletion of the critical region of the WHS (4p16.3), which is more distal from the telomere.
The infant was referred to us when she was 4 years old, with a developmental delay and a bone age showing a delay of two years. The high resolution G-band karyotype (850 bands) was normal. The application of the Multiprobe-T kit showed a subtelomeric 4p deletion. The application of Fluorescence in situ Hybridization (FISH) with the WHS syndrome probe, showed no chromosome deletion. Thus her karyotype was defined as: 46,XX,del(4p).ish(tel4p-/tel4q+).ish(p16.3)(WHSCRx2). Her parents showed no chromosome alteration including subtelomeric regions.
Although our case apparently has a very distal 4p deletion, not including the WHS critical region, presents clinical manifestations similar to that observed in the WHS. We consider that those cases with clinical appearance of WHS, with no deletion of the WHS critical region should also be analyzed for subtelomeric deletion.

P0181. A model for evaluation of maternal cell contamination in spontaneous abortions cultured cells: significance for cytogenetic analysis of prenatal selection factors

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Chromosomal abnormalities play a leading role in early human lethality, however, estimation of fetus karyotype by long-term cell culture may not be accurate because of possible contamination of such cultures by maternal cells. In the present study, we examined the results of retrospective cytogenetic analysis of 478 first trimester spontaneous abortions. Maternal cell contamination (MCC) was evaluated by PCR analysis of amelogenin gene for the presence of Y chromosome DNA in non-cultured cells of “46,XX” embryos. A mathematical model for the estimation of MCC level with expected rates of different karyotypes in abortions is proposed. The experimental validation of the model was performed by analysis of segregation of polymorphic microsatellite DNA markers for 6 autosomes in 60 “46,XX” embryos and their parents. In 16% of the embryos with a 46,XX karyotype a molecular assay has revealed Y chromosome DNA. Taking MCC into account, the frequency of chromosomal abnormalities in spontaneous abortions rose from 54.6% to 60.3% and the sex ratio in abortions with normal karyotype increased from 0.66 to 1.02. The model allows to evaluate the diagnostic accuracy of conventional cytogenetic analysis of reproductive wastages, which amounts to 92.3%. MCC clearly affects the incidence of chromosomal abnormalities and the sex ratio in spontaneous abortions. Thus, correction for MCC should be taken into account before invoking biological explanations of sex ratio bias, such as the effect of X-linked lethal genes or abnormal X-inactivation, and may be useful to include in diagnostic reporting.

P0182. Breakpoint characterization by FISH in a child with mild features of dup(3q) syndrome due to an interstitial inverted duplication 3q (q26.31q24) *de novo*.

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Dup(3q) syndrome is defined as a characteristic phenotype associated with duplications of 3q21-qter. Clinical evaluations suggest, that duplications involving a critical region in distal 3q26.3 is sufficient to produce dup(3q) syndrome. In this report we describe a 15-months-old boy with mild dysmorphic features, abnormal head shape and microcephaly. Craniofacial features of dup(3q) syndrome included low-set ears, upslanting palpebral fissures, a broad nasal root with anteverted nostrils, long philtrum, downturned corners of the mouth, micro-retrognathia and short neck. Further findings included a sinus pilonidalis, tapering fingers, small feet with syndaktylia 2/3 and bilateral sandal gap.
Classical cytogenetics by use of high resolution Q, G and R banding methods revealed a *de novo* structural aberration comprising an additional segment in 3q, the pattern of which appeared symmetrical to the adjacent one. By fluorescent *in situ* hybridization (FISH) using a whole chromosome painting probe (wcp 3) and arm specific probes (pcp 3p, pcp 3q) the additional material was shown to have originated from 3q. In order to define the breakpoints of this interstitial duplication, FISH with 33 BACs was performed. By this means breakpoints were narrowed down to 3q24 proximal and 3q26.31 distal. Proximally the breakpoint was assigned to a region smaller than 50 kb.
The mapping data of this duplication are compared with other cases defined by pure duplication in similar segments of chromosome 3q. We submit that relevant genes of dup(3q) syndrome lie in the region 3q26.2-q26.31.
The karyotype of the patient was delineated as 46, XY, dup(3)(3q26.31q24).

P0183. Somatic instability of a constitutional large ring chromosome 7 by nuclear extrusion and micronucleus formation

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Ring chromosome formation by fusion of the terminal chromosome arms is a well known structural aberration being documented for most human chromosomes. Beside sporadic somatic events a variety of cases displaying a constitutional ring chromosome either additional or replacing the respective normal chromosome are described in the literature. Surprisingly, although different chromosomes are affected, the reported individuals with numerically normal karyotype show phenotypic similarities as growth retardation, mental delay and microcephaly, leading to the suggestion of a ,,ring syndrome“. The specific ring syndrome phenotype in varying ring chromosome conditions has been attributed to functional impairment and increased apoptosis of cells caused by mitotic instability of the chromosomal rings.
We here report a rare case of a large ring chromosome 7 in a 14 year old growth retarded boy with severe mental delay, where we could demonstrate chromosomal instability in blood cells showing as well duplication but mainly loss of the structurally abnormal chromosome 7 by active nuclear extrusion and micronucleus formation. An increased micronucleus formation is frequently observed in exogenously induced chromosomal instability, however, has not been shown in a constitutional ring chromomosome condition.

P0184. An unusual reciprocal translocation detected by subtelomeric FISH: Interstitial and not terminal.

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An 11 month-old boy with a pattern of dysmorphic signs, an ASD, right inguinal hernia, bilateral undescended testes, bilateral urinary reflux and right renal dysplasia, and developmental delay revealed an abnormal chromosome 11 with enlarged terminal long arm in his karyotype. The paternal karyotype was normal 46,XY, while the mother showed the same 11q+ and in addition a 2q- chromosome. Chromosome paiting with chromosome 2 and 11 libraries confirmed a reciprocal translocation with a tiny 11 signal on 2q and a larger 2 signal on 11q. Thus, a reciprocal terminal exchange was assumed.The karyotype of the mother would thus be 46,XX,t(2;11)(q 35;q24.3). FISH with 2q and 11q subtelomeric probes was performed in order to confirm the reciprocity of the translocation. However, to our great surprise we did not find the expected pattern, namely an 11q signal on the rearranged 2 and a 2q signal on the rearranged 11. It turned out that the translocation was not an terminal reciprocal exchange, but was interstitial on both chromosomes which is highly unusual. Thus, the results „moved“ the aneuploid segments to a more proximal position, especially the deleted 11q segments since it does not contain the subtelomeric FISH region. The molecular cytogenetic results in this family show that reciprocal translocations should be investigated not only with whole chromosome paiting, probes but also with further probes for the subtelomeric region since, for genetic counseling, karyotype-phenotype correlation and gene mapping it is of increasing importance to determine the breakpoints and aneuploid segments as precise as possible.

P0185. Mosaicism for ectopic NOR at 8pter and complex rearrangement of chromosome 8 in a patient with severe psychomotor retardation

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We report on a 3-year-old girl with severe delays in mental and motor skills, absent communication, generalized seizures, and subtle dysmorphisms. The third child of a healthy non-consanguineous Caucasian couple was born at term with Apgar scores of 10/10 after 1 and 5 minutes. Birth weight (3000 g), length (51 cm), and head circumference (35 cm) were normal for gestational age. At the age of six months, developmental delay was first noticed. Metabolic screening showed no pathological changes. Cranial magnetic resonance imaging demonstrated a reduction of myelin mass but no malformations. Conventional cytogenetics revealed a mosaic karyotype. A de novo ectopic NOR at the telomeric region of the short arm of one chromosome 8 (8ps) was found in 90% of lymphocyte and in 98% of fibroblast metaphases. A derivative chromosome 8 without short arm satellites and a small NOR-bearing marker chromosome were present in the remaining cells. Der(8) included an inverted duplication of 8p and a rearranged duplication of 8q but lacked a second centromere. SKY analyses demonstrated that der(8) consisted entirely of chromosome 8 material and chromosome painting probes specific for 8p and 8q confirmed the rearrangement. FISH with a probe specific for centromeres 14 and 22 labeled both the telomeric region of 8ps and the small marker centromere. A subtelomeric probe for 8p revealed a cryptic deletion in 8ps and der(8). Since severe mental retardation and seizures rarely occur in mosaic trisomy 8 syndrome, the submicroscopic partial monosomy 8pter most likely contributes to the phenotype.

P0186. *De novo* formation of a Y neocentromere during complex rearrangements leading to a 47,X,r(Y),i(Y) karyotype

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An aberrant karyotype with 47 chromosomes including two different sized marker chromosomes was identified during prenatal diagnosis. Fluorescence *in situ* hybridisation (FISH) with a Y painting probe tagged both marker chromosomes, that were supposed to be isochromosomes of the short and the long arm, respectively. Both parents revealed normal karyotypes.
To characterise both Y markers in detail we FISH-mapped a panel of Y chromosomal probes originally developed to present a standard FISH-signal pattern for the human prometaphase Y chromosome (Roettger *et al.* 2002). This panel included probes for *SHOX* (PAR1), *TSPY*, DYZ3 (Y centromere), *UTY*, *KALY*, *RBMY*, *XKRY*, *EIF1AY*, *DAZ*, *CDY*, DYZ1 (Yq12 heterochromatin), *SYBL1* (PAR2) and the human-all-telomere sequence $(TTAGGG)_n$.
The smaller Y marker turned out to be a ring chromosome containing the entire short arm, the original Y centromere and parts of the proximal long arm, including AZFa. Size variations of this ring Y chromosome in different metaphases may be due to double or even multiple ring formation during mitosis. The bigger Y marker, indeed, was an isochromosome of the Y long arm. Surprisingly, hybridisation of DYZ3 detected no Y-specific alphoid sequences on it, despite of a clearly visible primary constriction within one of its DAPI-positive heterochromatic regions. Because of its stable mitotic distribution a *de novo* formation of a neocentromere has to be accepted.
Furthermore, we detected an interstitial deletion in Yp11.22 and Yq11.23 that must have occurred during the formation of this complex rearrangement. Thus, the karyotype of this boy may be described as 47,X,r(Y)(pterq11.21),i(Y)(qter—>q11.23::q11.22::q11.22::q11.23—>qter).

P0187. Supernumerary marker chromosome: cryptic chromosomal rearrangement resulting in partial trisomies 21 and 12

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We report on a cytogenetic investigation of a 2 year old boy without preliminary information of the childs phenotype.
Cytogenetic investigation (GTG-banding) revealed an aneuploid karyotype with an additional G-group chromosome indistinguishable from chromosome 21 (resolution 450 bands). However clinical information obtained from the referring pediatricians excluded any major features of Down syndrome.
The boy was born after an uneventful pregnancy at term beeing the first child of unrelated parents. At the age of 12 months epilepsy with myoclonic astatic seizures occured. Beside delayed motor and speech development at the age of two years, he shows slight dysmorphic features such as macrocephaly, rectangular face with prominent cheeks, hypertelorism, high forehead, short neck, small chin and zygodactyly 2/3.
Due to the lack of phenotypical features of Down syndrome, a FISH analysis with the Down syndrome region specific probe (LSI 21, Vysis) has been performed and revealed two signals on the two normal chromosomes 21, but no signal on the derivative chromosome 21.
The following Multiplex-FISH identified the supernumerary derivative chromosome 21 as the result of a cryptic rearrangement: translocation of the distal end of the short arm of chromosome 12 to the long arm of chromosome 21. The unbalanced rearrangement was further confirmed by chromosome painting probes (WCP12, WCP21, Vysis) and probes specific for the subtelomeric regions of chromosomes 12p and 21q.
Karyotype: 47,XY,+der(21)(21pter→21q22.12::12p13.3→12pter).ish der(21)(wcp12+,wcp21+,D21S259x2,D21S34x2, D21S342x2,VIJyRM 2029x2,VIJyRM2196x3) de novo.
This case demonstrates anew that preliminary detailed clinical data is indispensable for a correct diagnostic approach.

P0188. Reciprocal translocations: how many are complex rearrangements?

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We report 4 cases of reciprocal translocations, where FISH analysis

showed an unexpected complexity of the rearrangement.
The first patient is a 8-year-old girl with cranio-facial dysmorphisms, psycomotor and growth retardation, monolateral neuroblastoma.
FISH analysis with subtelomeric specific probes (kit Vysis) showed a semicryptic translocation, of maternal origin, between the long arm of chromosome 6 and the long arm of chromosome 15: 46,XX,t(6;15)(q 22;q21)mat.
Further FISH experiments with Bacs specific for 6q and 15q highlighted an insertion of the region 6q22.31-6q22.33 on the long arm of a chromosome 10 to the band 10q25.2.
The second case concerns a Prader-Willi patient with karyotype 46, XY,t(15;18)(q11;q22)de novo. FISH analysis showed the involvement of a third chromosome, one chromosome 21. Further analysis demonstrated that the der(21) was fragmentated in several portions translocated on the other two derivative chromosomes.
The third patient is a child with cerebral malformations and a karyotype: 46,XX,t(2;4)(p12;q31)de novo. FISH analysis demonstrated that 2p12-pter was translocated on two non adjacent regions of chromosome 4q: 4q25 and 4q28 and that the region containing the breakpoint of the der(2) was inverted. Furthermore, the der(4) showed an interstitial deletion of about 20Mb on the short arm.
The last patient is a young boy with facial dysmorphisms, psychomotor retardation, micropenis and a karyotype: 46,XY,t(7;10)(p12;q24)pat.
FISH analysis demonstrated a duplication of 10q24-q25 in the propositus but not in the father.
These data stress the need to investigate reciprocal translocations with molecular analysis to allow a correct understanding of the rearrangement.

P0189. An interstitial deletion on chromosome 13 in a boy with mental retardation and bilateral retinoblastomas.

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The patient was born at term, no. 2 of 2 by non-consanguineous parents. He was mild hypotonic at birth with a large occipital cranium. The boy continued to be hypotonic and at 5 months he could not hold his head. Further investigations were done and an examination of the eyes shows bilateral tumours in the retinas. This was confirmed with MRI showing retinal tumour, increased space in the subarachoidal room bifrontally and mildly increased sulci. The retinoblastomas were treated with radiation.
Standard chromosome analysis displayed a short 13q.
Due to the retinoblastoma FISH with locus specific probe for RB1 was carried out (13q14.2). There was only one signal from the locus supporting the suspicion of a deletion including the RB1 gene. Multicolour Banding FISH confirmed the breakpoints at 13q14.1 and 13q22.
Cytogenetic and molecular genetic results will be presented.

P0190. Subterminal deletion/duplication event in an affected male due to maternal X chromosome pericentric inversion

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We report a 13-month-old male infant with severe growth and developmental delay, ichthyosis, hypogonadism, limb shortness, hypoplasia of the corpus callosum and a round, flat face and thin upper lip as a consequence of a recombinant X chromosome (rec(X)) derived from crossing-over within the inversion identified in a family. On routine karyotyping the patient's chromosomes were considered to be normal, with pericentric inversion of the heterochromatic region of chromosome 9. Because of the phenotypic abnormalities, FISH was performed using subtelomeric probes. On one X chromosome two signals for Xqtel were detected on each tip of the chromosome, while there was no signal for Xptel . The patient's father's chromosomes were normal, but his mother had pericentric inversion of chromosome 9 and one abnormal X chromosome. Pericentric inversion of the X chromosome was confirmed by FISH analysis. Further FISH analysis of the patient and his mother's chromosomes using BAC and YAC probes revealed the breakpoints on the X chromosome. The rec(X) had a duplication of the segment Xq27.3 →Xqter and deletion of the Xp22.31→Xpter. We concluded that the patient's karyotype was: 46,Y,rec(X)(qter→q27.3::p22.31→ qter),inv(9)(p11q13).
The proband's phenotype corresponds to descriptions of contiguous gene syndromes due to deletion of STS, SHOX, ARSE and KAL genes. Despite the loss of the ARSE gene there is no evidence of chondrodysplasia punctata. Additional conditions associated with disomy of the Xq28 segment, such as severe growth and developmental delay and a peculiar shape of the head and mouth, were observed.

P0191. Subtelomeric translocations in couples with recurrent miscarriages

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Introduction Unbalanced chromosomal rearrangements involving telomeres are emerging as an important cause of mental retardation and congenital malformations in humans. As suggested by several authors, they could also be responsible for recurrent miscarriages. This chromosomal rearrangements escape diagnosis by conventional cytogenetic methods. So, it is necessary specific techniques for detecting subtelomeric translocation.
Objectives The aim of this study was to investigate the presence of subtelomeric translocation in couples with recurrent miscarriages.
Materials and Methods This study was performed in eighteen clinically normal couples who have had four or more spontaneous abortions and whose karyotypes were found to be normal using conventional cytogenetic techniques.
Fluorescence in situ Hybridisation (FISH) with specific probes of chromosomal telomeric regions (ToTelvision TM Multi-Color, VYSYS) to metaphase spreads was used.
Results Among the 36 individuals studied, only one subtelomeric translocation was detected between 2p and 3p chromosomes. The female carrier of this translocation had had seven miscarriages and no viable offspring.
The rest of the couples showed normal results.
Conclusion Subtelomeric translocations are not a usual finding among couples with recurrent miscarriages. The high economical and labour costs of these studies make its clinical application impractical.

P0192. Partial trisomy 16q due to a maternal balanced translocation t(16;21)(q22;q22.3) in a boy with multiple malformations: further delineation of the trisomy 16q phenotype

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Partial trisomy (16)(q22-qter) represents a rare condition. Only a few patients with familial rearrangements are described (A. Schinzel: Catalogue of unbalanced chromosome aberrations in man, 2nd edition, 2001: 685). We report a dysmorphic boy with a partial trisomy 16q due to a maternal balanced translocation t(16;21)(q22;q22.3). The chromosomal aberration [karyotype: 46,XY,der(21)t(16;21)(q 22;q22.3)] was detected at postnatal diagnosis and confirmed and defined by GTG/QFQ banding in the parents and fluorescence in situ hybridisation (FISH) with whole chromosome paints and subtelomere probes for chromosome 16 and 21. The boy, who showed intrauterine growth retardation was born by Caesarean section in the 36th gestational week (weight 2500g, length 43cm, head circumference 33 cm, Apgar 9-10-10). The following dysmorphic features were observed on physical and clinical examination after birth: trigonocephaly, hypertelorism, strabism, conspicuous hearing test, long philtrum, thin lips, high palate, small mandible, unilateral choanal stenosis, wide spaced nipples, patent ductus arteriosus, single palmar creases, proximal placement of thumb, hypoplastic scrotum and cryptorchid testes. The boy suffers from dysphagia and failure to thrive and shows a general developmental delay. Comparison of our patient with other cases presenting partial trisomy 16q allows a further delineation of the dup(16)(q22-qter) phenotype and a precision of the phenotypic map of chromosome 16q.

P0193. Contribution of FISH to the detection of subtelomeric rearrangements in syndromic mental retardation

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Introduction Subtelomeric rearrangements are a cause of syndromic mental retardation. Fluorescent in situ hybridisation (FISH) techniques with telomeric probes can detect alterations in these regions. However, not all the rearrangements detected will have phenotypic manifestations.
Objectives Search for subtelomeric rearrangements among patients with syndromic mental retardation and to asses whether the alterations detected are pathological by complementary molecular and familiar studies.
Materials and Methods Sixteen patients with moderate-severe mental retardation and congenital abnormalities were selected. FISH technique with telomeric probes was performed on metaphase spreads. Those cases with alterations were analysed with polymorphic markers in order to delimit the affected regions. Familiar studies were also carried out in these cases.
Results Two cases with a subtelomeric imbalance were detected. One case showed a derivative chromosome 6 with loss of the most terminal 6p and gain of 3qter. The other case showed a derivative chromosome 5 with loss of pter and gain of 9pter. Both derivatives were inherited and familiar studies pointed out the pathological status of these imbalances.
Conclusions The study of cryptic chromosomal imbalances offers a lot of advantages. In addition to offer genetic counselling to the affected families, it will allow us to know more about the genetic causes of mental retardation. Moreover the alterations detected could be the starting point for the characterisation of new genes.

P0194. Micronucleus Frequency in Rat Embryonic Blood Cell Treated with Anticoagulant Agents

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Thromboembolic complications during pregnancy are the most common causes of maternal and fetal morbidity and mortality. Heparin and low-molecular-weight heparins (LMWH) are used to reduce the incidence of venous thromboembolism in pregnancy and improve outcomes. LMWH products are dalteparin, enoxaparin and nadroparin.
In the literature, cytogenetic studies in animals have been evaluated the genotoxic potential of these products. There were not any mutagenetic effect in in vivo rat bone marrow chromosomal aberration and micronucleus (MN) frequency. However, there is no published data about the effects of LMWH on the cells of developing rat embryos. In this study, we aimed to analyse genotoxic and/or cytotoxic effects of anticoagulant agents on the MN formation in rat embryonic blood cells. Rat embryos were exposed to the different clinically relevant concentrations of anticoagulant agents (heparin 5-40 iu/ml; dalteparin 2.5-15 iu/ml; enoxaparin 25-100 µg/ml; nadroparin 1-4 iu/ml) for 48 hours in culture between 9.5 and 11.5 days of embryological development in which early organogenesis takes place. After culturing the embryos, their blood samples were collected by removing the visceral yolk sac and the embryo in RPMI-1640 medium. The samples were prepared according to classic MN assay. It was observed that the increased concentrations of the used agents caused a dose-dependent increase in the MN frequency in rat embryonic blood cells when compared to MN frequency in controls. This result indicates that the possible genotoxic and/or cytotoxic effects of anticoagulant agents on the developing rat embryo.

P0195. The assessment of genotoxicity of chloral hydrate in newborn lymphocyte culture

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Chloral hydrate is a sedative commonly used in pediatric medicine. It was evaluated for genotoxicity in cultured peripheral blood lymphocytes of newborns administered chloral hydrate for sedatization. Sister chromatid exchanges and micronucleus frequencies were measured before and after chloral hydrate administration. Sister chromatid exchanges and micronucleus were significantly found to increase after administration. The results of the study suggest that chloral hydrate might have a moderate genotoxic potential in newborns.

P0196. FISH analysis in children with developmental delay, dysmorphism and malformations

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Chromosome aberrations are frequent cause of developmental delay, dysmorphism and congenital malformations. Cytogenetics has low resolution power and chromosome anomalies smaller than 5Mb cannot be detected. FISH is useful method for detecting submicroscopic rearrangements. In this report we present the results of FISH study in 140 children with developmental delay, dysmorphism and congenital malformations (DDM). The FISH method with microdeletion probes and ToTelVysion multi-color FISH panel for subtelomeric screening was performed according to the manufacturer's suggestions (Vysis). Microdeletions were detected in 14(11.0%) out of 127 children with phenotype suggestive of microdeletion syndromes. FISH analysis revealed hemizygosity for 22q11.2 in 7(6.7%) and re-evaluation revealed deletion of ELN locus in 2(1.95) additional patients out of 104 suspected for DiGeorge/VCFS. Williams syndrome was diagnosed in 3(37.4%) out of 8 patients, Angelman in 1, and deletion of SNRPN locus in 3(50%) out of 6 children referred for Prader-Willi syndrome. Subteromeric FISH revealed no rearrangement in 9 patients with DDM and normal karyotype and contributed to the precise characterization of 4 investigated structural chromosomal aberrations with suspected involvement of telomere. This report confirms that FISH is powerful method for diagnosis of microdeletion syndrome. Although no rarrangement was detected by subtelomeric screening in this small group of patients, this study points that multisubtelomere FISH is useful in understanding the mechanism of origin of structural rearrangements, and emphasize the need of selection criteria and precise phenotype evaluation of the patients prior to subtelomeric testing. Supported by the Ministry of Science of the Republic of Croatia (TP-01/072-01).

P0197. ECARUCA: an internet-based European database for collection of rare chromosomal abnormalities

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During recent years a considerable improvement in diagnostic techniques has enabled cytogeneticists to find more and smaller chromosomal aberrations. However, accurate clinical knowledge about rare chromosome disorders is frequently lacking, mostly due to a significant decline in published cases. On the other hand, there is an increasing demand from parents and physicians for reliable information about the disorder of their child or patient.
Therefore, we established the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA). This internet-based database, funded by the European Union, collects cytogenetic and clinical data of (non-) published cases with a rare chromosome abnormality.
Currently, (cyto) genetic centres can submit data to the register by using the downloadable forms from the ECARUCA website, www.ecaruca.net . It will be possible to submit data by a web-based process soon.
The availability of collected data is subdivided in a two-level system: each internet user can view general information about a specific chromosome aberration and the accompanying clinical features. Whereas, account holders can receive specific data per case, including additional research and clinical pictures.

The collection and exchange of clinical and technical knowledge will allow for accurate information on clinical aspects of rare chromosome disorders that can be used by the professionals involved. Moreover, correlation study of chromosome aberrations and their phenotypes will be invaluable for localizing genes involved with mental retardation and congenital anomalies.During the presentation, the website and its possibilities will be demonstrated as well as our plans to optimise the communication of cytogenetic know-how within Europe.

P0198. An extremely rare case of reciprocal translocation homozygosity

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A homozygous reciprocal translocation, 46,XY,t(10;11),t(10;11), was detected in a 4 year old boy with congenital non-syndromic neurosensory hearing impairment. Both of his parents, who are consanguineous, carry the translocation in a heterozygous state and hear normally. The single translocation was also found in the couple`s daughter and their two other sons, who are all phenotypically unremarkable. The pedigree is consistent with an autosomal recessive form of deafness caused by disruption or otherwise inactivation of a gene in the breakpoint region(s). By chromosome banding, the breakpoints were located in 10q24.3-q25.1 and 11q23.3. Fluorescence in situ hybridisation of fully integrated BAC maps to patient chromosomes was used to localize the breakpoint on chromosome 10 to a 2 Mb interval and the breakpoint on chromosome 11 to a 1.5 Mb interval, respectively. The breakpoint region on chromosome 11 is associated with an autosomal recessive locus (DFNB24) for hearing impairment and is therefore particularly interesting. Positional cloning of the breakpoint regions is underway to uncover a novel gene(s) involved in congenital hearing loss.

P0199. De novo 9-break-event in one chromosome 21 combined with a microdeletion in 21q22.11 in a mentally retarded boy with short stature

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We report on a moderately mentally retarded 12-year-old boy of short stature showing the most complex chromosomal rearrangement (CCR) within a single chromosome ever described. A de novo derivative chromosome 21 was recognized in GTG-banding shortly after birth. However, the nature of the rearrangement remained obscure up to the application of the chromosome 21 specific centromere-near multicolor-FISH (subcenM-FISH; Starke et al., 2003; Hum Gene. 114:51-67) probe set and of six selected locus-specific probes along chromosome 21. An unbalanced 9-break-event was uncovered with breakpoints in 21p13, 21p12-13, 21q11.2, 21q21.1, 21q22.11, 21q22.11, 21q22.12, 21q22.22 and 21q22.3. A deletion of 21q22.11 was detected by application of the BAC probe bk249H10. The karyotype can be described as 46,XY,der(21)(:p13->p12~13: :q22.3->q22.22::q11.2->p12~13::q11.2->q21.1::q22.11->q21.1:: q22.12->q22.22::p13->p13). The clinical signs can either be due to gene inactivation in connection with structural changes at the break and fusion regions, to the building of new fusion genes within the CCR and/or to the deletion of genes in 21q22.11. Supported in parts by the Dr. Robert Pfleger-Stiftung and the DFG (PO284/6-1).

P0200. Structural rearrangements of chromosome 3 in two children with mental retardation

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In this paper we present two cases with mental retardation and dysmorphic features in whom structural rearrangements of chromosome 3 were identified by cytogenetic studies.
In the first case, a rare chromosomal abnormality consisting of an interstitial deletion of long arm of chromosome 3, was found in a 5-year old girl with mild mental retardation. Phenotype features included cranial and mandibular anomalies and congenital cerebral malformation. Cytogenetic analysis, performed on GTG-banded chromosomes, identified an interstitial deletion del(3)(q22) Proband's parents and sister have normal phenotypes and there was no abnormality revealed by cytogenetic studies. Thus, the interstitial deletion of the chromosome 3 was considered "de novo".
The second case, a 4-year old girl was referred for cytogenetic analysis with severe mental retardation. She had facial dysmorphic features and hyperkinesia. The chromosomes studies revealed a duplication dup(3)(p22p24). Beside duplication 3p22-3p24 an unbalanced translocation between the long arms of chromosomes 5 and 9 was identified in a single metaphase. Proband's parents are phenotypically normal.
Two rearrangements involving the chromosome 3 were identified in two children with non-syndromic mental retardation. In order to elucidate the mechanisms responsible both for the interstitial deletion of the long arm and for the duplication of the short arm of chromosome 3 molecular techniques are required.
Acknowledgements. National Program VIASAN, Project 127/2001-2003.

P0201. Distal monosomy 11q and partial trisomy 20q in a girl with MCA/MR.

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Terminal deletion of 11q is a distinct clinical entity, also known as Jacobsen syndrome. Frequent features include mild to moderate psychomotor retardation, cardiac defects, thrombocytopenia and facial dysmorphism including trigonocephaly, telecanthus / hypertelorism, broad depressed nasal bridge, micrognathia and low-set dysplastic ears. The deletion classically described is del(11)(q23→qter), but breakpoints may varying. Terminal 20q trisomy is a rare chromosomal anomaly; all reported cases to date were associated with another chromosomal anomaly.
We present a 2-year-old girl with pre- and postnatal growth deficiency, a cardiopathy (aortic stenosis with hypoplasia of the aortic arch), left-sided hydronephrosis due to pyelourethral junction stenosis, frequent respiratory infections, psychomotor retardation and facial dysmorphism. Standard and high resolution chromosome analysis were normal. The diagnosis could be established by MLPA (Multiplex Ligation dependent Probe Amplification) analyses showing a diminished (1/2) intensity at telomere 11qter (Salsa probe 1143-L0700, KIAA1030 gene) and an increased intensity corresponding with telomere 20qter (Salsa probe 1091-L0642, URKL1/FLJ20517 gene). These results were confirmed by FISH analysis showing only one 11qter signal and three 20qter signals (one on both chromosomes 20 and one signal at chromosome 11q). More detailed DNA analyses revealed that the deletion occurred at the paternal derived chromosome and that the breakpoint is situated between markers D11S4151 and D11S934. Further studies to delineate the breakpoint are ongoing. Phenotype-genotype correlation will be discussed.

P0202. Subtelomeric chromosome aberrations as a cause for miscarriages? FISH screening of 30 samples from pathological pregnancies

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Subtelomeric chromosome aberrations have been implied as a possible cause for recurrent miscarriages. Several studies of couples with more than three abortions pointed to a low overall prevalence of *familial* subtelomere aberrations (3 out of 348 patients, summarised in Cockwell et al., Hum Genet 112 (2003): 298-302). To our knowledge, a systematic study of pathological pregnancies themselves has not been performed so that no data are available about the incidence of *de novo* subtelomere aberrations.

We report on a study of 30 samples from pathological pregnancies from gestational weeks 9 to 36. In order to exclude an ascertainment bias towards familial aberrations, samples were collected consecutively including all karyotypically normal samples with sufficient metaphase numbers. Screening was performed using a novel combinatorial Subtelomere-Six Colour-FISH with BAC and PAC probes mainly from the second generation probe panel published by Knight et al (Am J Hum Genet 67 (2000): 320-332).
29 out of 30 subtelomere screenings gave normal results suggesting a low incidence of subtelomere aberrations in miscarriages.
In the remaining case an enlargement of 22p diagnosed after amniocentesis had been misinterpreted as a maternal heterochromatic polymorphism. A large euchromatic intrachrosomal 22q duplication of at least 7 Mb was identified by Subtelomere-Six Colour-FISH (46,XY,der(22)(qter→q13.2~q13.1::p12~p11.2→qter). The breakpoint characterisation using a panel of BAC and PAC FISH probes is ongoing.

P0203. Multicolor chromosome banding (MCB) in the interphase nucleus

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All up to present published FISH studies on the interphase architecture are performed on interphase nuclei which are prepared using the airdrying procedure of chromosome preparation. This standard method and similar approaches (Solovei et al., 2002; in: FISH – a practical approach; Oxford-Univ-Press: 119-157) lead to a flattening of the nuclei or produces, in the best case, a half-spherical shape. When interphase architecture shall be studied this may lead to questionable results. Recently we described a technique, where the whole FISH-procedure can be performed on cell suspension and the cells are brought on a concave polished slide as the final step of the procedure, just before the evaluation. We called this procedure suspension-FISH = S-FISH (Steinhaeuser et al., 2002; J Histochem Cytochem 50: 1697-1698) and demonstrated that the nuclei maintain their spherical nature here. In this study we used the S-FISH approach to do MCB based 3-D-analyses on spherical interphase nuclei derived from peripheral blood of normal controls and bone marrow cell suspension. A multicolor banding (MCB) probe set for chromosome 5 was applied in 3 clinical cases with MDS or AML with known deletions in chromosome 5q of different sizes ('5q- syndrome'). We were able to detect the known chromosomal rearrangements in the interphase nuclei. The breakpoints could be confirmed as well. Thus, we demonstrated the possibility to detect and describe chromosomal alterations on interphase nuclei.
Supported in parts by the "Förderverein des Klinikums der FSU Jena e.V." and the EU (QLRT-1999-31590/QLRT-2000-01590).

P0204. A complex rearrangement of chromosome 1q associated with a distinctive phenotype and cerebellar malformation

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Cerebellar hypoplasia may occur as an isolated abnormality or as part of a multiple anomaly syndrome. It is often associated with a posterior fossa cyst, the two abnormalities being referred to as the Dandy Walker malformation. Cerebellar hypoplasia may arise due to a single gene disorder or chromosome aberration. We report the case of a 5 year old girl with delayed development, dysmorphic features, atrial septal defect, atrophy of the corpus callosum and cerebellar hypoplasia in association with a small corpus callosum and a pineal cyst. Banded chromosome analysis showed an apparently balanced inversion of 1q32.3-42.1. Further analysis using BAC-FISH revealed the rearrangement to be more complex including two separate inversions and two deletions of 3-4Mb. Cerebellar malformations have not previously been reported in association with aberrations of 1q. The deleted regions may hold a gene, or genes with an important role in cerebellar development.

P0205. Complex chromosomal rearrangements - cytogenetical and clinical data in Belarus Registry of Chromosomal Abnormalities.

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Complex chromosome rearrangements (CChR) may cause decreased fertility, reproductive failure, abnormal offspring. We studied a spectrum and clinical data of CChR detected by GTG-banding, FISH methods among patients with malformations, mental retardation, hypogenitalism, amenorrhea, miscarriages. 45 patients with balanced or unbalanced CChR, inherited and sporadic, were found.
I group - balanced CChR (15 patients) presented: combinations of reciprocal or Robertsonian translocation with inversion; two inversions 46,XX,inv(8)(p11.2q11.2),inv(9)(p11q12)14pss,15p+ and 46,XY,inv(7)(p11.1q21),inv(9)(p11q13), two translocations 45,XX,t(2;20)(q21;q13),der(14;21)(q10;q10). Phenotype abnormalities were noted in 3 patients with CChR(dn): 46,XX,inv(8)(p11.2q11.2),inv(9)(p11q12)14pss,15p+ and 46,XX,inv(2)(p23q37),t(5;12)(q11.1;q22) - mental retardation, multiply malformations. 45,XX,der(15;15)(q10;q10),inv(9)(p11q12) - mental delay, severe obesity. In 2 cases of CChR patient inherited each aberration from each parent: 46,XX,t(6;16)(p23;q22)mat; inv(2)(p11q13)pat; and 46,XX,t(3;5)(p14.2;p15.3)mat; inv(9)(p11q12)pat.
II group - unbalanced CChR (30 cases):
1. Inbalance of autosomes (16 cases):
Down syndrome (DS; 11 patients with typical phenotype) is due to combinations of full trisomy 21 with inherited balanced translocation - t(11;21)(q21;q22)mat, t(12;22)(p11.2;q13)pat, or inversion - inv(7)(p12q21.1)mat; inv(9)(p11q13).
Mosaic karyotypes: 47,XY,+21/48,XY,+21,+21, and 48,XY,+21,+mar/46,XY.
Rare forms: 46,XY,t(3;12)(q13.2;q24.2),del(10)(p12)dn; 48,XY,+mar,+mar; 47,XX,t(11;22)q25;q13)pat,+del(22)(q13); 47,XX,+8,inv(9)(p11q12)/46,XX,inv(9)(p11q12)pat; 46,XX,der(2),t(2;11)(q36;q22)mat, inv(9)(p11q13).
2. Inbalance of gonosomes (9 cases):
Turner syndrome is due to combination of monosomy X or structural abnormality X with translocation or inversion. Klinefelter syndrome - 47,XXY with inherited balanced t(3;14)(p21;q24) and der(13;14)(q10;q10) or inv(9)(p11q13). Rare variants-47,XYY,t(6;15)(p213;q24)/45,X,t(6;15)(p213;q24)mat; 46,X,del(Y)(q11), inv(9)(p11q12)dn.
3. Inbalance of autosomes and gonosomes (5 cases): combination of DS and Klinefelter syndrome – 48,XXY,+21 and 47,XXY,der(14;21)(q10;q10),+21mat. Rare forms (dn) – 47,XX,+18[90]/45,X[10] and 48,XXX,+ider(9)(p10)/47,XXX.
Cytogenetical and clinical data of CChR will be presented. Problems of genetic counseling for families with CChR will be discussed.

P0206. Skin manifestations in mosaic trisomy 16 syndrome

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Trisomy 16 is the most frequent chromosome abnormality at conception and the most common autosomal trisomy in spontaneous miscarriages. Nonmosaic trisomy 16 has been described in very few cases that survived to third trimester and seems to be incompatible with postnatal survival. Trisomy 16 mosaicism, however, has been described in CVS as a common abnormality. The clone with trisomy 16 might be confined to placenta or identified at birth in fibroblasts and/or lymphocytes. Variable clinical expressions of this mosaicism have been described. Intrauterine growth retardation seems to be the rule and characteristic abnormalities include asymmetry of the skull or limbs, congenital heart defects and eye abnormalities. To the best of our knowledge there are no reports of follow up beyond the first few years of life and only in one patient skin abnormalities have been described.
A case of distinctive, unilateral dermatological manifestations characterized by hypertrichosis, telangiectasia, hyperkeratosis and slight hyperpigmentation in a 48-years-old man is presented.

Additional findings were left-sided skeletal abnormalities and hearing loss. Analysis of fibroblasts from two different samples of affected skin showed trisomy 16 mosaicism. A normal chromosome copy-number was found in blood lymphocytes and fibroblasts from unaffected skin.
The majority of mosaic trisomy 16 pregnancies originate from maternal nondisjunction in the first meiotic division followed by 'trisomic zygote rescue' whereby a chromosome 16 is lost. The rescue may result in uniparental disomy. The importance of uniparental disomy will be discussed. Based on the clinical characteristics a possible relation to Becker naevus syndrome is suggested.

P0207. Frequency and spectrum chromosomal aberrations in somatic cells at the population living in a zone of influence of Semipalatinsk test site

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Purpose: To study cytogenetical effects in somatic cells in a population irradiated as result of ground and atmospheric nuclear tests in 1949-1962. We analysed the frequency and types of chromosomal aberration in 66,068 G-banded metaphases in 174 cultures of peripheral blood lymphocytes.
The greatest frequency of cells with aberrations (3.2±0.4%) is found in a zone of extreme radiation risk (ERR). This is double the frequency seen in the zone of minimal radiation risk (MRR) -(1.8± 0.2 %) and three times that in a control area -(1.04± 0.16 %).
Acentric fragments are the most prevalent aberration, with frequencies 1.3±0.2 per 100 cells in the ERR, 0.94±0.13 in the HRR, significantly higher than the MRR (0.43±0.06) and control zone (0.2±0.06).Dicentrics were seen at a frequency of 0.18±0.03 and rings at 0.27±0.06 per 100 cells in persons from the ERR and HRR, compared to 0.02±0.01 in persons from the MRR and control group.
The frequencies of radiation-influenced stable markers (deletions, translocations) were 0.74±0.16 (ERR), 0.84±0.12 (HRR), 0.63±0.13 (MRR) and 0.37±0.08 per 100 metaphases, respectively.
Thus, analysis of the frequency and types of chromosomal aberrations has shown that in somatic cells of the inhabitants of the surveyed areas the tendency to accumulation of unstable and stable radiation markers is observed, which testifies to possible continuation of the influence of radiation on chromosomal integrity.

P0208. Results of cytogenetic studies in 112 patients of the south of Tunisia investigated for infertility and reproductive failures

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The aim of this study was to evaluate the contribution of chromosomal abnormalities in infertility and reproductive failure.
We investigated cytogenetically 73 infertile men, 14 women with amenorrhoea and 10 couples who had 2 or more spontaneous abortions or recurring Art failure. Wives of 5 infertile men with OAT were investigated before ICSI. 34 infertile men were also analyzed for Y microdeletions.
The overall frequency of chromosomal abnormalities was 19,6% (22/112) and the prevalence of DAZ microdeletions in infertile men was 8,8 % (3/34).
Numerical sex chromosome abnormalities were more frequent than structural chromosomal rearrangements.
Frequency of cytogenetic abnormalities detected among infertile men was 19% attributable mainly to the inclusion of klinefelter's syndrome: 10 cases of 47,XXY karyotype. Excluding this category, only 6,3% infertile men were found to have chromosomal abnormalities: 1 case of de la chapelle syndrome [46,XX], 1 case of structural chromosomal rearrangements [45,XY,t(13;14)] and 2 cases of cytogenetic deletion of the Y chromosome. Chromosomal abnormalities in infertile women affected by primary or secondary amenorrhoea were found in 50% of cases. Chromosomal abnormalities concern exclusively X chromosome: structural in 2 cases and numerical (mosaic Turner's syndrome) in 5 cases.
Chromosomal aberrations in recurrent abortions occurred in only one couple of 10: a reciprocal translocation t(1;4).
High number of infertile subjects is affected by chromosomal aberrations. Routine cytogenetic analysis should be performed to explain infertility but also to counsel couples before ART. Screening of Y chromosome microdeletions is also recommended in men with severe male factor infertility.

P0209. Subtelomeric 9p;12q Rearrangement in a Family Passed Through Three Generations With Two Affected Individuals Presenting Unbalanced Karyotype

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We report on an unbalanced rearrangement of the telomeric regions of the short arm of one chromosome 9 and the long arm of one chromosome 12, karyotype 46,XY,der(9)t(9;12)(p24.3;q24.33) in a child of the age of 5 $^{3}/_{12}$ years, who presented with mental retardation and some dysmorphic features. Since conventional chromosome analysis alone did not reveal the rearrangement, the translocation was detected only by straightforward analyses combining cytogenetic and molecular cytogenetic methods including microdissection and telomere FISH analysis. Further studies revealed not only that the child's mother and grandmother were carriers of the 9;12 translocation but that the sister of the child's mother, who was reported to have mental retardation as well, carried the same unbalanced translocation as the child.
A partial trisomy 12q and a partial monosomy 9p due to a translocation 9p24.3 and 12q24.3, resulting from a maternal balanced translocation has been reported once in the literature in a newborn who passed away at the age of 2 ½ months. However, even if the breakpoints given appear to be similar to our case according to nomenclature, the rearrangement was detected by cytogenetic analysis already suggesting that the imbalance of the chromosomal regions may be more serious.
We present the clinical, chromosomal and molecular cytogenetic findings of the two affected individuals and other family members investigated so far, comparing our results with the available literature data.

P0210. The evaluation of genotoxic potential of a nitroimidazole, ornidazole, in lymphocyte culture of patients with amebiasis

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The genotoxicity study of ornidazole was carried out on human lymphocyte chromosomes using sister chromatid exchange and micronucleus. 22 patients with *Entemoeba histolitica* infections who received 1000mg/d for 10 days participated in this study. Sister chromatid exchanges and micronucleus were measured before therapy and after therapy. A statistically significant increase was observed in the SCE (Z= -5.778, p<0.001) and MN frequencies (Z= -5.712, p: 0.004) after ornidazole therapy could be described. It was concluded that ONZ has a potential genotoxic and cytotoxic effect in human peripheral lymphocyte cultures. Further and detailed studies are needed to elucidate ONZ mechanism of genotoxicity and its carcinogenic potential, since the drug is widely used.

P0211. FISH detection and characterisation of subtelomere aberrations in 76 mentally retarded patients - High diagnostic yield among mildly affected patiens

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The aetiology of mental retardation (MR) which affects 2-3% of the population is unknown in about half of the cases. Subtelomeric chromosome aberrations have on average been determined in 4,6% of patients with idiopathic MR (Xu and Chen, Am J Med Genet 117(2003):15-24).

We performed FISH screening for subtelomeric chromosome aberrations in 76 clinically well documented MR patients with normal banded karyotypes. Subtelomere FISH was carried out using two different probe sets and three techniques. The first probe set was a commercially available one (ToTelVysion™, Vysis). The second set consisted of BAC and PAC probes mainly from the “second generation probe panel. The latter one was applied in two different multi-colour FISH techniques: Subtelomere-COBRA-FISH and Subtelomere-Six Colour-FISH.
Subtelomere aberrations were detected in 5 patients (6,6%). Both unbalanced translocations [der(2)t(2;5)(q37.3;p15.1), der(2 2)t(7;22)(q36.3;q13.33)] and all three deletions [del(14)(q32.31), del(2)(q37.3), del(16)(p13.3)] had occurred de novo. The breakpoints of the aberrations were characterised by FISH using panels of BAC and PAC probes in order to contribute to karyotype-phenotype correlations. With special regard to the clinical preselection “checklist” proposed by de Vries et al. (J Med Genet 38(2001):145-150), we compared the phenotypes of our patients with and without subtelomere aberrations. Most of our patients with subtelomere aberrations had relatively mild phenotypes and displayed only few of the selection criteria of the “checklist” and an implementation of the proposed cut-off would have excluded three out of five aberration carriers, demonstrating the need for further discussion of selection criteria.

P0212. Chromosomal Abnormalities in Recurrent Miscarriage. A preliminary study of 250 Romanien couples

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A proportion of cases with repeated abortion are caused by chromosomal abnormality in one of the parents
This study includes 250 couples with recurrent miscarriage and/or stillborn infant who were referred for cytogenetic studies between July 2000 and December 2003 at two of the Genetics Center of Timisoara. Cytogenetic features of peripheral blood lymphocytes cultivated according to standard techniques on PB MAX medium for 72 h were evaluated. The GTG analysis revealed the karyotypes.
The number of previous abortions varied from 2 to 14 abortions (mean 3 abortions/couples). In 42 (8,4%) of these cases chromosome abnormalities were identified.
These abnormalities includes: 5 cases with robertsonian translocations [t(13;21), (21;22), (14;14)], 2 cases with balanced reciprocal translocations [t(2;6), t(6;7)]; 8 cases with pericentric inversions [inv(9), inv(16)]; 4 cases with duplications [1(dup) and 16(dup)]; 2 cases with [22p+, 15p+] ,1 case with insertion ins(3); 1 case with r(13), 2 cases with deletions del(Yq); mosaic forms of gonosomal aneuploidies in 7 cases, structural anomalies of X chromosome [inv(X), iso(X), del(Xp), del(Xq)] in 4 cases, 15 satelite cases.
Polymorphisms: 1qh+(3cases), 9qh+(26cases), 13ph+(1case), 16qh+(2case), Yqh+ (1case) were seen in 13,2 %.
Genetic counseling is suggested for each pregnancy loss. After the second pregnancy loss the chromosome analysis of the conception product and of both parents must be taken into account. If some abnormal chromosomes are detected in one or both of the parents, the amniocentesis must be performed in order to determine the fetal karyotipe in each future pregnancy.
Kay words: recurrent miscarriage , abnormal chromosomes.

P0213. Chimerism analysis with FISH and PCR methods

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The term chimerism refers to the presence of lympho-hematopoietic cells of non host origin. Full or complete chimerism (CC) generally refers to complete replacement of host by donor lymphohematopoiesis mixed chimerism (MC) indicates the presence of both donor and recipients cells within a given cellular compartment. The two tests most widely used today are in situ hybridization and PCR-based methods. Both methods have a high sensitivity and spesificity.
In this study, patients were being followed up at Istanbul Medical School , BMT unit between 1999-2003. Ten patients with acute leukemia were evaluated for chimerism between 21th day and 18th month after allo BMT . We used FISH method and PCR amplification and reverse dot blot. We performed FISH assay with CEP X/Y probe (Vysis) to 10 patients.The Amplitype PM Kit includes PCR amplification reagents for following six genetic loci Low Density Lipoprotein Receptor, Glycophorin A , Hemoglobin G Gammaglobulin , D7S8 and Group Spesific Component .
Patients had an average age of 31.5±8.7 and the ratio of male to female was 4:6. Eight patients were shown to be in state of complete chimerism with both methods. Two of all patients were shown to be in a state of mixed chimerism with PCR analysis. One of the mixed chimeric patients was found to be complete chimeric by further FISH of analysis .
X/Y probes were useful with all patients who had received a transplant from a sex-mismatched donor. PCR-based method is also very rapid and efficients at discriminating siblings.

P0214. Pericentric inversion of chromosome 2, 46,XX,inv(2)(p13;q37), associated with recurrent abortions - A new case?

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Familial pericentric inversions of chromosomes, 9 and Y are relatively common and are considered to be normal polymorphic variant in the general population occurring with varying frequencies. However, *de novo* pericentric inversions of some chromosomes are causally associated with infertility, reproductive failure, multiple congenital anomalies, dysmorphic features, due to recombinant aneusomy. We report here a new case of pericentric inversion of chromosome 2, in a female spouse of an Indian couple investigated for history of 3 repeated first trimester abortions. Chromosome analysis using standard Giemsa trypsin banding technique showed, 46,XX, inv(2)(p13;q37). Karyotype of the husband was normal 46,XY. A review of literature suggests that pericentric inversions of chromosome 2 increases the risk for spontaneous abortions but do not increase the risk for unbalanced recombinant aneusomy for chromosome 2. The pathogensis of pericentric inversion 2 will be briefly reviewed.

P0215. Cytogenetic studies in the referred population of Dubai, United Arab Emirates -1986-2002: A report of 14,546 cases

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We report here cytogenetic findings seen over the last 16 years (1987-2002). A total of 14,546 cases were investigated for suspected chromosomal abnormalities, at the Genetics Department of Al Wasl Hospital, DOHMS, Dubai (UAE), providing diagnostic genetic services to seven emirates states in the UAE. Aneuploidy of the autosomes and the sex chromosomes are relatively common as in other similar studies. Chromosome analyses of GTG banded metaphases among 14, 546 cases showed, trisomy 21in 579 cases, followed by trisomy 18 in 55 cases, trisomy 13 in 21 cases. Among sex chromosome aneuploidy, 45,X (Turner syndrome) was the most common seen in 39 cases, followed by 13 cases of 47,XXY, 5 cases of 47,XXX. 1 case of 48,XXXX and 1 case of 49,XXXXY. Structural abnormalities were also observed, including 124 case of translocations, 16 cases deletion and 3 duplication. Ring chromosome was detected in one case(ring 2). This study gives an idea of spectrum of chromosomal abnormality in the heterogeneous population of Dubai (UAE). The data is compared with similar studies in the Arab population.

P0216. ISCNAnalyzer easily detects karyotype errors in cytogenetic databases

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CyDAS (Cytogenetic Data Analysis System) is planned to become a

comprehensive integrated tool for the analysis of cytogenetic data. As a first step, we developed a class library for the evaluation of ISCN karyotypes. This ISCNAnalyzer was tested with karyotypes from the Mitelman Database of Chromosome Aberrations in Cancer (by kind permission of F.Mitelman).
Of 22445 entries dating from 1995 or later, 20140 (90%) were parsed without problems. Errors were recorded in 2305 datasets. About half of the errors were due to incomplete descriptions of aberrations, e.g. missing breakpoints (977, 42%) or missing aberration descriptions for derivative chromosomes (270, 12%). Such information could often be obtained from other karyotypes of the respective publication. Incomplete descriptions also often reflected problems to achieve a more detailed characterization of complexly aberrant karyotypes. Most interestingly, 420 (18%) entries showed breakpoints in not-existing chromosome bands. Moreover, non-acceptable breakpoint designations of translocations or dicentric chromosomes were used in 200 cases (9%), and other errors in derivative chromosomes were found in 266 (12%) records.
In the Mitelman Database, karyotypes of published data are recorded as written in the publications irrespective of typewriting mistakes or other errors. Therefore, our findings indicate that karyotype data need to be checked more closely before publication and that the ISCNAnalyzer may be a useful tool for finding faulty data in cytogenetic databases.
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P0217. Case report: boy with severe hypotonia, hypogenitalism, chronic interstitial pneumonia, and partial X disomy, karyotype 46,X,der(Y)t(X;Y)(q28;q11.2)

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Aberrant Xq-Yq interchange in the father's germline can lead to Xq DNA present on a truncated Y chromosome. The patients have partial X disomy and abnormal gene expression resulting from the absence of X inactivation. The phenotype (severe mental retardation, generalized hypotonia, microcephaly) was named X-Y(Xq) syndrome (Lahn et al., Nat Genet 1994;8:243-250). Few patients have been described to date. We report a term-born boy, birthweight 3100g, length 50 cm, and Apgar 8/9. He failed to thrive, had feeding difficulties and frequent episodes of pneumonia. Cystic fibrosis was strongly suspected but excluded by molecular studies. Findings at 13 months included moderate mental retardation, short stature (-3 SD), microcephaly (-3.5 SD), flat occiput, round face, hairy forehead, broad eyebrows, almond-shaped eyes, blue sclerae, strabism, full cheeks, micrognathia, low-set dysplastic ears, short neck, broad thorax, wide-spaced nipples, single palmar crease, and hypogenitalism (micropenis, cryptoid testes, hypoplastic scrotum). The chorionic gonadotrophin test showed no increase of testosterone (0.69 nmol/l, normal range 8-38). Cytogenetic and FISH studies revealed a translocation of Xq28, karyotype 46,X,Yq-.ish der(Y)t(X;Y)(q28;q11.2)(DYZ3+,DXYS14+,DYS221-,*MTM+*,*L1CAM+*,*SYBL1+*). Thus, the boy had disomy Xq28-qter and nullisomy Yq11.2-qter. He died 18 months old with chronic interstitial pneumonia.
Nullisomy and disomy Xq28 in boys share many clinical manifestations, including the retardation, microcephaly, seizures, and hypotonia, possibly due to inappropriate expression of *FLN1* (MIM 300049). In our patient, we hypothesize that the hypogenitalism and the chronic interstitial pneumonia, respectively, could possibly be caused by overexpression of *F18* (a candidate gene for genital development) and *BAP31/DXS1375E* (an inhibitor of CFTR expression).

P0218. A rare chromosomal aberration: partial trisomy of 2q35-q37.3

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Duplications of chromosome 2q are uncommon and mostly originate from balanced familial aberrations. Consequently, partial trisomy 2q often occurs along with a monosomy or trisomy of another chromosome segment. We present a 17-year-old girl with an isolated duplication of a small distal segment of chromosome 2q. After normal motor development, there was an arrest in the mental development. Growth and weight followed the 97th centile, head circumference was normal. She had brachycephaly, a square face with protruding eyes, a beaked nose, clinodactyly on both hands, and 2nd rays of the feet were shortened. Chromosome analysis revealed an altered chromosome 2q with additional material. Parents were not available for analysis. By FISH with chromosome 2 specific library, a subtelomere probe for 2q, and a probe generated by microdissection of the additional segment (Micro-FISH), the diagnosis of partial trisomy 2q35-q37.3 was established. About 20 cases have been reported with isolated duplications of various regions of 2q. To our knowledge, only two of them had a comparable, but not identical, partial trisomy of 2q. The delineation of a distinct phenotype of distal trisomy 2q remains difficult because malformations are rare and growth patterns are variable. With increasing age, more characteristic facial signs (plagiocephaly, square face, beaked nose, buphthalmus) develop and might allow to define a common phenotype. Constant findings are mental retardation, clino-, camptodactyly, and genital abnormalities in males. Besides adding to the clinical characterisation of distal trisomy 2q, our case further illustrates the usefulness of Micro-FISH in the elucidation of chromosomal rearrangements.

P0219. Partial Deletion of the Long Arm of Chromosome 13 in a Fetus with Dandy-Walker Malformation Supporting Previous Observations of a Candidate Gene Loci

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Dandy-Walker malformation (DWM) is a genetically heterogenous entity that can present as an isolated malformation or in association with genetic syndromes, single gene disorders, chromosomal abnormalities or other multifactorial conditions.
The present case was terminated after DWM, and limb defects were noted on ultrasound at 21 weeks gestation. The autopsy findings at 22 weeks gestation revealed right renal agenesis, lobe anomaly in the lung (two lobes on the right, an incomplete fissure on the left), and only four digits on all four extremities in addition to absence of cerebellar vermis, a posterior fossa cyst continuous with the fourth ventricle, and hydrocephalus which confirmed DWM.
The chromosome analysis of cord blood showed 46,XY, del(13) (q14-q34).
Malformations of the central nervous system and other anomalies have been described with deletions of the long arm of chromosome 13. One of the critical regions for holoprosencephaly (HPE) is now known to be located on this region. McCormack et al. have reported two cases with HPE and DWM with deletion of the long arm of chromosome 13 suggesting that a haploinsufficiency at a locus within 13q22-33 may cause this anomaly. One of the patients described also had 4 digits on each extremity similar to our case.
Our findings support the possibility that the candidate gene loci for isolated DWM may be found in this particular region. We also suggest careful cytogenetic examination in patients with DWM.

P0220. Small reciprocal insertion detected by spectral karyotyping (SKY)

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Insertions are rare, reciprocal insertions most unusual. Reciprocity of insertions has been reported only in three patients. We observed the exchange of small fragments between chromo-somes 4 and 16. A now six year-old boy presented with insufficient opposition of the hypoplastic thumbs, thenar hypoplasia, brachydactyly, single palmar creases, cryptorchidism, epicanthal folds, an enlargement of the cavum septum pellucidum, a spinal dermal sinus (i.e., spina bifida occulta), muscular hypotonia, and borderline mental retardation. Hearing test and ophthalmologic examination were normal. The

karyotype was unbalanced, 46,XY,der(16)ins(4;16)(q27q28.2;q 12.1 or q13) pat. In the father a reciprocal insertion, 46,XY,rep ins(4;16)(q27q28.2;q12.1 or q13), was found. The father also had brachydactyly but his thenars and thumbs were normal. He had bilateral hearing loss of about 50% which, however, was attributed to frequent otitis media during childhood.
The chromosomal origin of the insertion in chromosome 16 of the propositus and its balanced reciprocity in the father were identified by spectral karyotyping (SKY). Cryptic and half-cryptic interchromosomal insertions in the size range of 1 - 5 Mb can be detected by routine cytogenetics if hybrid-ization techniques such as SKY are implemented. In the individuals presented here, FISH analysis with BAC clones is being applied now to characterize the deletion/ insertion borders. If the rearrangement comprises the chromosomal region 16q12.1, Townes-Brocks syndrome is to be diagnosed in the son resulting from haploinsufficiency of *SALL1* which is located in this region.

P0221. Y; autosomal translocation or cryptic tissue mosaicism in a boy with 45, X0?

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Sexual differentiation of males is determined by the presence of the Y chromosome. More precisely, in order for phenotypically male genitalia to develop, the presence of the Sex-determining Region gene on the Y chromosome (SRY) is neccessary. The presence of the SRY gene has been always detected in males, irrespectively of their karyotype apart from exceptionally rare cases. Recently, we have examined a 15 year old boy with hypogonadism but normal male genitalia (testicles approximately 2ml bilaterally), gynecomastia and mental retardation (IQ=54). LH, FSH and testosteron studies showed prepubertal levels in the patient. Repeated karyotypic analysis of peripheral lymphocytes revealed a 45, X0 chromosomal arrangement in all of the 150 cells examined. The same karyotype was found in the fibroblasts of the patient. No sign of mosaicism was detected by interphase FISH of the centromeric region of the Y chromosome (Yp11.1-q11.1) in the blood or buccal mucosa. Additionally, no signal suggesting the presence of Y material on any chromosome could be detected in the peripheral lymphocytes by FISH with Y painting probe. Considering the phenotypic appearance, the results of gonadal histology, conventional and FISH cytogenetic analyses, we suggest that apart from translocation of the SRY containing region of the Y, cryptic tissue mosaicism in some cell lines could also be the causative factor of the pathology in this patient.

P0222. The Clinical Spectrum of Pericentric Inversion of Chromosome 8 in a Saudi Family

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Recombinant 8 syndrome is a well established clinical entity characterized by mental retardation, and severe cardiac anomalies. The majority of the reported cases so far have been of Hispanic origin. Here, we report a 14 years-old Saudi boy with a paternally inherited pericentric iinversion of chromosome 8, inv(8)(p23.1q24.22). He presented with microcephlay, short stature and abnormal genitalia, but otherwise a normal cognitive function. Both of his identical twin brother as well as his father are phenotypically normal. His 8 month old brother was diagnosed prenatally on amniocentesis with rec(8)dup(8q)inv(8)(p23.2q24.22). He was small for gestational age, mildly hypotonic, had aterial septal defect and ambiguous genitalia.
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P0223. Cytogenetic analysis of spermatozoa îf 45,XY, der (13;14) patient after their injection into mouse oocytes.

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Robertsonian translocation is one of the most common chromosome rearrangement in humans. Carriers of Robertsonian translocations are usually attributed with male infertility (oligiastenoteratospermia) and have an increased risk of spontaneous abortions and newborns with genetic abnormalities.
To assess frequency and type of chromosome aberration of vital spermatozoa from patient 45,XY, der (13;14) with severe oligoastenoteratospermia heterologous ICSI fertilisation of mouse oocytes with human spermatozoa was used. The spermatozoa from fresh or cryopreserved-thawed sample were applied.
In total 57 suitable metaphase plates of human sperm chromosomes were analysed. Frequency of normal spermatozoa (26,31%) is not different from frequency of balanced spermatozoa (40,36%) (χ^2 test, P>0.05). Ratio of X- to Y-bearing spermatozoa is not significantly different from the expected 1:1 in normal and chromosomal balanced spermatozoa (χ^2 test, P>0.05). Frequency of hyperploid spermatozoa was 10,52%, frequency of spermatozoa with and without der(13;14) was equal - 5,26%. Frequency of spermatozoa with additional chromosome 13 and 14 was 3,50 % and 0%, respectively. Frequency of spermatozoa with nullisomy for chromosome 13 and 14 was 5,26% and 3,50 % respectively. Frequency of spermatozoa with structural abnormalities was 14,03% (8,77% spermatozoa with der(13;14) and 5,26% without it). Thus there was no any interchromosomal effect and preferential disjunction of translocation with gonosomes, additional or rearranged chromosomes (χ^2 test, P>0.05).
Despite of the modest sample size our data suggest that chromosome analysis of spermatozoa from carrier of chromosome rearrangements can give more information about proportion of normal and abnormal spermatozoa. This information will be useful for genetic counselling.

P0224. Single cell analysis by sequential application of two different approaches: multicolor interphase FISH followed by single cell CGH

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Genome analyses of single cells become increasingly important in different applications.
In clinical diagnostics single cell- approaches are essentiell e.g. in cases of mosaicisms. Furthermore, sophisticated single cell analysis may enable the examination of fetal cells in maternal blood to realize a non- invasive prenatal diagnosis. In cancer, single cell analyses are needed especially for monitoring minimal residual disease and for the assessment of genetic changes within disseminated tumor cells (micrometastasis). Here we demonstrate the sequential analysis of individual cells by two different single cell techniques. After interphase FISH cells are selected based on the number of FISH signals, isolated by laser microdissection, amplified by linker adapter PCR and analysed by CGH. Examples will be presented for normal controll cells and for tumor cells with multiple copy number changes.
The combination of these two different techniques allows an efficient control of the single cell CGH results through interphase FISH.

P0225. Generation of chromosome painting probes from single chromosomes by laser microdissection and linker-adaptor PCR

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Fluorescence *in situ* hybridization (FISH) plays an essential role in research and clinical diagnostics. The versatility and resolution of FISH depends critically on the used probe set. Here, we describe a novel approach for the generation of specific DNA probes from single copies of chromosomes. Single chromosomes or single chromosomal regions were microdissected by laser pressure catapulting and amplified using linker-adaptor PCR. The probes were labeled and tested in various scenarios including multicolor-FISH experiments employing up to seven different fluorochromes. FISH confirmed the specific and evenly staining of the respective chromosomal regions. Furthermore, the capability of these probes to detect even small

translocations (<3 Mb) suggests that the dissected regions are completely represented in the generated painting probes.

P0226. Subtelomeric screening - a method to solve family mysteries

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20 years ago a couple (T) asked for counselling because of several mental retarded siblings to the woman. Cytogenetic analyses for the couple (T) and two still alive affected brothers of the woman have been performed with normal results.
Couple T got three children, a son and two daughters. One daughter (AT) showed mental retardation and presented at age of 12 years at our institute. Again cytogenetic analysis was normal. The other daughter (HF) is healthy and has two healthy children. The unaffected son (ST) married and got two daughters of whom the first-born (DT) showed mental retardation. The second girl is healthy. During third pregnancy of his wife the family presented again at our institute in 2003.
We performed subtelomeric screening for AT and DT and identified in both the same subtelomeric unbalanced translocation t(6;22)(q27;q13.3)(tel6q-,22q13.3+). Consecutively we examined blood samples of two still alive mental retarded brothers of Mrs. T and found also the same above mentioned translocation. Several mental healthy family members were identified as carriers for the balanced form of the above mentioned translocation, e.g. ST as well as HF and her children. At last prenatal diagnostic via amniocentesis in the third pregnancy of ST´s wife revealed the specific subtelomeric unbalanced translocation in this family. As the fetus would be most likely affected by mental retardation pregnancy was interrupted.
This family history shows that cases of unexplained mental retardation should be reexamined especially via subtelomeric screening to solve family mysteries.

P0227. A Cytogenetical Study of Pediatric Necropsies at an Universitary Hospital With a Selective Clinical Methodology

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This study has used a selective methodology for detection of chromosomal anomalies in pediatric necropsies (died between 20 weeks of gestation and 15 years old). We had 1.558 pediatric necropsies including 464 with congenital anomalies . The cytogenetical study was obtained in 94 children with isolated congenital malformations (from 211 = 44.5%) and no chromosomal anomaly was detected in this group and, in 179 with multiple congenital anomalies (from 253 = 70.8%) and where were detected 72 chromosomal anomalies in this other group= 28.5%.
The total frequency of chromosomal anomalies was 4.62% and if we consider only the perinatal death, this frequency was 3.1% (26 chromosomal anomalies in 835 necropsies). These frequencies are similar to non-selective samples (3.5 to 4.4% - Machin and Crolla, 1974; Bauld et al, 1974; Kuleshov, 1976; Angeli et al, 1984; Sutherland and Carter, 1983).
If we consider only perinatal death, we had 17 cases of autosomal trisomies (18, 13 and 9) = 65.4%; 6 cases of structural anomalies = 23 %; 2 cases of sexual reversion = 7.7% ; and 1 case of triploidy = 3.8%. Kuleshov, 1976 reported that in non-selective studies of chromosomal perinatal mortality 55% were by autosomal trisomies, 20% by sexual chromosome anomalies and 20% by structural chromosome anomalies. The main difference between our selective sample work and the non-selective was that there was no detection of sexual chromosomal anomalies. The detection of autosomal anomalies were very similar.

P0228. Partial trisomy 2p13->22: A case report

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We report a male term newborn with growth retardation (<p3) and low birth weight (p3) but normal head circumference (p25). A broad and high forehead, hypertelorism, small, low set posterior rotated dysplastic ears and micrognathia were noted. Other symptoms were small tongue, glossoptosis, cleft palate, syndactyly of fingers and hypospadia. Sonography of the brain showed dilatation of posterior horn of lateral ventricles and large cavity of septum pellucidum. Pulmonary artery stenosis was suspected. The karyotype showed extra material on the short arm of chromosome 2. Parental karyotypes were normal. Painting probes suggested that the extra material was derived from chromosome 2. An interstitial duplication 2p13->p22 was suspected and confirmed by CGH.
We reexamined the boy at the age of five months. Severe growth retardation with particularly short limbs and macrocephaly was noted. MRT showed hydrocephaly, partial agenesis of callosum, hypoplastic limbic gyrus and hypoplastic hypophysis. The boy had a tracheostoma and a gastrostoma. Other clinical findings were nephropathy, anal stenosis and recurrent hyperinsulinemia. Profound developmental delay with hypotonia was present.
To our knowledge an interstitial duplication 2p13->22 have not been described before. We will compare the symptoms of our patient with other cases with interstitial duplication 2p. Our case helps to further delineate the phenotype of this chromosomal aberration.

P0229. Duplication of chromosome 8p23.1p11.2 in two unrelated families.

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Tandem duplications are direct or inverted duplications of genetic material, ordered one after the other. Inverted duplications of chromosome 8p (inv dup 8p), are relatively common structural rearrangements. The majority of intrachromosomal or interchromosomal duplications are presumably de novo and the recurrence risk is <0.5%. The duplication comprises chromatin of the same chromosome and results in trisomy of the segment concerned. We report here two cases of similar duplications referred for chromosome studies. G-banded metaphases analysed from the probands from phytohaemagglutinin (PHA)-stimulated peripheral blood lymphocytes using standard cytogenetic techniques revealed an inverted duplication of the short arm of chromosome 8 resulting in partial trisomy for the segment p11.2 → p23.1.
Case 1: A 5-month-old baby boy presented with hypotonia, poor head control, agenesis of the corpus callosum and congenital heart defects.
Case 2: A 6-month-old baby girl with failure to thrive and some dysmorphic features. She was the third child conceived through in-vitro fertilization and has two normal, healthy siblings. Both parents are phenotypically normal and non-consanguineous.
In both cases, parental blood for chromosome studies were recommended. Case 1, the parents were not done and the origin of the abnormality was unknown. In Case 2, an apparently normal karyotype was observed in both parents suggesting a de novo interstitial duplication in the child.
More documentations of patients are needed to clarify the exact clinical significance of duplication of distal end of 8p.

P0230. Expanding the basic genetic defect in Wolf-Hirschhorn syndrome

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Wolf-Hirschhorn syndrome (WHS) is a MR/MCA condition caused by partial 4p deletion. Although genotype-phenotype correlations mostly depend on the extent of the deletion, unexplained phenotypic variability is a typical hallmark of WHS. The genomic defects represent a de novo event in most cases. De novo rearrangements are largely assumed to be isolated deletions. We analysed a total of 37 WHS families. Molecular cytogenetics was carried out with 4p-specific cosmid or BAC clones (total 70) and with all subtelomeric probes. We found that 32 (86.5%) out 37 rearrangements were de novo, 5 (13.5%) segregated from a balanced parental translocation. Among de novo rearrangements, 21 (66%) were isolated 4p deletions, 11 (34%) were double chromosome anomalies, in particular unbalanced de novo translocations (N=7), involving chromosomes 8p (5 cases), 11q and 7p, inverted 4p duplications associated with

terminal deletion (N=3), intrachromosomal recombinant (N=1).
The parental origin of the deleted chromosome 4 was paternal in 83% of cases (25/30), maternal in 17% (5/30). Paternally derived rearrangements were usually isolated deletions (21/25), double anomalies being unfrequent (4/24). Maternally derived rearrangements all were unbalanced de novo t(4p;8p) translocations (5/5).
Double chromosome anomalies were first mistaken as cryptic deletions (less than 3.5 Mb), causing apparent genotype-phenotype inconsistencies. On the contrary, the actual deletion size was 12 Mb (N=5), 5 Mb (N= 4) and 20 Mb (N=1).
The different categories of the basic genomic defect in WHS identify specific clinical phenotypes.
These observations can be considered a model for haploinsufficiency syndromes affecting different chromosomes.

P0231. Molecular cytogenetic breakpoint analysis in three familial cases of Xp;Yq translocation

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Xp;Yq translocations are very rare, and molecular characterization suggests their etiology through aberrant exchange between homologous sequences on Xp and Yq. Males with Xp;Yq translocations are usually nullisomic for a portion of Xpter and their phenotype depends on the extent of the Xp deletion. They may present chondrodysplasia punctata (CDPX), short stature or Léri-Weill syndrome (SHOX), mental retardation (MRX), ichthyosis (STS) and Kallmann syndrome (KAL). The phenotype of females carrying Xp;Yq translocations with the corresponding deletions of Xp material is usually normal, except for short stature.
We have performed molecular cytogenetic breakpoint analysis in three familial cases of Xp;Yq translocation. In two families the Y chromosomal breakpoint was situated adjacent to the AZFa distal boundary within KALY (Yq11.21), while the X chromosomal breakpoints are within STS (Xp22.31) in one and within KAL (Xp22.31) in the other family. In this latter family the sole male carrier suffers from developmental delay and mental retardation with the molecular exclusion of fra(X) syndrome. In the third translocation family the breakpoints differ: the breakpoint on the Y chromosome is located adjacent to the AZFa proximal boundary (Yq11.21), and the breakpoint on the X chromosome is located distal to ARSE very close to the pseudoautosomal boundary in Xp22.33. X-inactivation studies using the BrdU-replication technique revealed preferential inactivation of the Xp;Yq translocation chromosome in blood lymphocytes of female carriers in all three families studied. The manifestations in male and female with specific loss of Xpter and gain of Yq material are discussed.

P0232. The evolutionary history of human chromosome 7

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We report on a comparative study on evolutionary changes of human chromosome 7 homologs using a combined molecular cytogenetic and in silico approach to reconstruct the succession of rearrangements in all major primate lineages. The ancestral primate homologs to chromosome 7 comprised of two chromosomes (7a and 7b/16p). From these the segment 7a was further derived by a paracentric inversion as shared by lemur and higher Old World primates. The ancestral higher primate chromosome form was further derived by a fission of 7b/16p and a centric fusion of 7a/7b as observed in the orangutan. Two further inversions with four distinct breakpoints were described in detail: a pericentric inversion in the African ape ancestor and the subsequent paracentric inversion in the common ancestor of human and chimpanzees. FISH analysis employing BAC probes confined the 7p22.1 breakpoint of the pericentric inversion to 6,8Mbp on the human reference sequence map and the 7q22.1 breakpoint to 97,1Mbp. For the paracentric inversion the breakpoints were found in 7q11.23 between 76,1Mbp and 76,3Mbp and in 7q22.1 at 101,9Mbp, respectively.
All four breakpoints were flanked by large segmental duplications. Hybridization patterns of breakpoint flanking BACs and the nature and distribution of duplicated segments suggest that they have been present before the origin of both inversions. We propose a scenario of the origin of both inversions by which segmental duplications may have been the cause rather than the result of these chromosome rearrangements.

P0233. Refinement Of The X-chromosome Breakpoint In A Female Patient Carrying A 4;x Translocation.

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We present molecular genetic studies of a female patient affected with Choroideremia, primary amenorrhea and mild sensorineural deafness who has been already reported (Lorda-Sanchez et al.; 2000) showing a balanced translocation between chromosomes 4 and X. Previous cytogenetic analysis revealed that the breakpoint in the X chromosome was in the Xq21 region. The gene associated with Choroideremia (CHM) lies within the X- chromosome breakpoint. The CHM gene is localised on Xq 21.2 and the 15 exons identified span a genomic sequence of about 150kb that encodes an ubiquitously expressed protein of 653aa called Rab Escort Protein-1 (REP-1). Different mutations including large genome rearrangements involving the REP-1 gene are responsible for Choroideremia disease but all of these cause truncated or absent protein.
We carried out two different techniques in order to refine the X-chromosome breakpoint.
1- FISH was performed with specific CHM gene probes: 103D (hybridises to 5 ´UTR and exón 1) and 293 (hybridises to exons 14 and 15 and 3´UTR).
2- Expression levels analyses of different CHM gene exons were carried out at cDNA level with specific pairs of primers previously described (van den Hurk et al.; 1997).
FISH analysis showed that the CHM gene was disrupted in the X-chromosome involved in the translocation and the expression levels analyses allow us to know that the breakpoint was localized between 13 exon and 14 exon of the CHM gene. Our results explain the CHM clinical picture showed by this female patient.

P0234. Deletion 4p of a der(4)t(4p;22q) in a newborn with a father carrying a balanced 4;22 translocation.

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A newborn girl was referred for chromosome analysis because of multiple congenital abnormalities. She was born at term with a birthweight and head circumference below the 2nd centile. She had downslant of the eyes, blepharophimosis, a high prominent nasal bridge, micrognathia, atrial septal defect and abnormal pulmonary venous return. Moreover, one kidney was missing. The girl died after six days. The parents had had one spontaneous abortion and the grandparents two miscarriages and a stillbirth at 6 months of pregnancy.
Chromosome analysis (GTG-banding) of peripheral blood showed a terminal deletion of the short arm of chromosome 4 (46,XX,del(4)(p1 6.1)).The father was found to carry a balanced translocation involving chromosome 4 and chromosome 22 (46,XY,t(4;22)(p16.1;q13.1)), which was also present in the grandfather. The chromosomes of the mother were normal. To characterize the breakpoint on 4p in the girl and her father, FISH with BAC clones was performed. BAC clone RP11-29H20 hybridized with both 4p and 22q in the father; mapping the translocation breakpoint within this clone. In the girl a signal with BAC RP11-29H20 was observed on the del(4). However, this signal had a lower intensity compared to the signal on the normal chromosome 4. No signal was observed on 22q. Molecular studies demonstrated that the „deleted“ chromosome 4 in the girl originated from the translocation chromosome 4 in the father. Probably, an adjacent-1 segregation during spermatogenesis resulted in a derivative (4)t(4;22) and one normal chromosomes 22 in the daughter and an additional event caused the partial deletion of the der(4) chromosome.

P0235. FISH studies of autosomal supernumerary marker chromosomes (SMCs)

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FISH studies of autosomal supernumerary marker chromosomes (SMCs)
The cytogenetic and phenotypic abnormalities of 26 patients (9 females and 17 males) aged 8 days -15 years, with autosomal SMCs are presented. With the use of FISH we were able to identify the origin of the SMC in 21/26 (80.7%) cases. In 9 patients the SMC originated from chromosome 15 (42.8%), in 5 from chromosome 15 (23.8%), in 2 from 13 (9.5%) and in 3 from chromosomes 1,8,22. In 2 unrelated cases the SMC contained material from both chromosomes 11 and 22, resulting from 3:1 miotic non-disjunction,since in each family one of the parents was a carrier of t(11;22). In 2 other cases the SMC was familial, as it was also present in a phenotypically normal parent, in 19 it was de novo and 3 families were unavailable for investigation. The majority of cases showed the SMC in all metaphases studied (19/26), while in 7 it was found in a mosaic form, ranging from 3% to 50%, The identification of the SMC allowed correlation with the clinical phenotype and genetic counseling of the families.

P0236. Unexpected malformations in a female fetus with a deletion Xp: demonstration of a cryptic translocation by MLPA

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A woman was referred at 31 weeks gestational age because of fetal growth retardation. On fetal ultrasound a heart defect and double bubble, indicating duodenal atresia, were seen. Amniocentesis was performed and an unbalanced female karyotype with a deletion of the short arm of the X-chromosome was found: 46,X,del(X)(p22.2). FISH with a paint Xp showed fluorescent signals on the entire aberrant Xp. Both parents had normal karyotypes.
At 39 weeks a girl was born weighing 1496 grams. Duodenal atresia and heart malformation were confirmed and in addition she appeared to have a cleft palate with micrognathia, ectrodactyly of the right hand, corpus callosum agenesis and hypoplastic kidneys. During the neonatal period a central diabetes insipidus became apparent.
Directly after birth EDTA-blood was obtained from the umbilical cord for Multiplex Ligation-dependent Probe Amplification (MLPA), which identified a duplication of 19p. FISH analysis with a subtelomeric probe for 19p confirmed this result. Thus the girl had a cryptic unbalanced translocation: 46,X,der(X)t(X;19)(p22.2;p13.3).
Duplications of 19p are extremely rare. This is most probably due to the gene richness of chromosome 19 and due to the difficulty to demonstrate small duplications of this negatively staining chromosome. The two cases described before had some features in common with our patient: growth retardation, micrognathia, heart defect and hypoplastic kidneys. The severe phenotype in our patient is remarkable since a skewed X-inactivation in favour of the normal X-chromosome would be expected. We are currently performing X-inactivation studies in our patient.

P0237. Two unrelated children with partial deletion of chromosome 7q

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Among aproximative 30 examples of interstitial deletion involving 7q no recognisable clinical syndrome was decribed (Gorlin, 2001).
We report two unrelated children, 1 month respectively 14 years old; the parents are healthy and nonconsanguineous. The patients were born following an uncomplicated pregnancy.
Clinically, the patients have in common growth retardation, microcephaly, broad nasal bridge with bulbous tip, down slanting palpebral fissures, dysplastic ears, and micrognathia.
The one-month-old patient has congenital heart defect (Fallot tetralogy). The karyotype showed an omogen 7q partial deletion 46,XY,del7(q32q33). To detect the origin of the deletion we recommended the karyotype for parents.
The 14 years old patient had a mild mental retardation. The patient's karyotype was 46,XX,del7q(34). The mother and two sister karyotypes were normal, so we can consider a de novo deletion on the patient.
We consider useful to present comparative the clinical features for the two patients.

P0238. Abnormal X chromosome in two patients with Turner syndrome

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Turner syndrome (TS) is a chromosomal disorder due to a complete or partial absence of an X chromosome. TS females classically manifest short stature, webbed neck, cubitus valgus and gonadal failure. Most TS features are the result of reduced dosage of X linked genes.
We report two patients, total different from clinically point of view, with structural anomalies of X chromosome.
One of them, 40 years old, with secundar amenorrhoea, short stature and poorly developed secondary sexual characteristics showed a mosaicism 45,X/46,X,delX(q22-qter). She has no cardiac or renal anomalies but has an autoimmune thyroiditis.
The 2 years old patient, with short stature, dysmorphic features, cardiac anomaly (VSD, pulmonar stenosis), moderate hydrocephaly and mild mental retardation, left ureterocel has an omogen karyotype 46,X,delX (p11.2-p11.4). The other 2 proband's sisters are clinically normal.
As the patients are clinically different due two distinct and rare interstitial deletions we consider the cases open for discussions.

P0239. Late-onset diseases among carriers of balanced chromosome rearrangements - results of a multicentre survey

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Large-scale breakpoint analyses in patients with disease-associated balanced chromosome rearrangements (BCRs) have enabled us to identify numerous gene defects underlying monogenic diseases. Late-onset and complex disorders are an even more promising target for this approach, given the multitude of genetic risk factors thought to predispose for such disorders. To ascertain patients with BCRs and complex diseases, we have performed a multicentre survey among adult carriers of BCRs using questionnaires inquiring about all aspects concerning health and disease. 141 carriers (aged 22-77 years, mean age 42 years) from 6 institutes returned the questionnaires. A causative role of the BCR was assumed if the rearrangement co-segregated with the disease in families, or if one breakpoint was located in a chromosomal region known to harbour

a gene for this disorder. Several patients met these criteria, as listed below:

Disease	Karyotype	Position of mapped locus, or co-segregation
Dyslexia	46,XY,t(5;16)(p15;q11)	Co-segregation in family
Hyper-IgE-Syndrome	46,XY,t(4;22)(q21.1;q12.2)	4q21 and co-segregation in family
Goitre	46,XX,t(7;14)(q32;q32)	7q31 and co-segregation in family
Psoriasis	46,XY,t(2;4)(p25;q31.1)	4q31.1
Schizophrenia	46,XX,inv(1)(p32q42)	1q42 (DISC1 and DISC2)
Epilepsy	46,XX,t(1;5)(p31;q22)	1p31
Cataract	46,XY,inv(9)(q22.33q34.1)	9q22
Stomatocytosis	46,XX,inv(9)(q22.1q34.1)	9q34.1 (STOMATIN gene)
Anaemia	46,XX,t(2;9)(p16;q13)	2p16 and 9q13
Endometriosis	46,XX,t(6;17)(q27;q25)	6q27 and 17q25

In view of the estimated 80,000 BCR carriers in Germany and the notorious disadvantages of association studies and related strategies, these initial results underline the potential of this approach for identifying genes for complex disorders.

P0240. A Down patient with chromosome 21 duplication

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Down syndrome is the most common chromosomal disorder, and is due to trisomy for all or a large part of chromosome 21.
We report a patient, 5 months old, with the characteristic phenotype of Down syndrome, cardiac anomaly (ASD, VSD), and a defect of the lacrimal system. She is the third child of an nonconsanguineous and clinically normal couple. The couple has another girl nine years old, clinically normal, a boy who died six hours after birth and a spontaneous misscariage at 3 months gestation. The patient was born after an uncomplicated pregnancy. The delivery was at 8 months gestation, APGAR 8, birth weight 2400g, birth length 45 cm. The karyotype showed an non-mosaic mirror image duplication 21q: 46,XX,dup21q. We recommended karyotyping of both parents and the older sister, which are now underway.
Down syndrome is only seldom caused by partial trisomy 21 resulting from a duplication of 21q, making our case an unusual one.

P0241. Tandem duplication of proximal chromosome 22q: clinical and molecular cytogenetic characterization

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We report on a 5-months-old girl with a de novo tandem duplication of the proximal part of chromosome 22. After intracytoplasmatic sperm injection dizygotic twins were born in the 36th week of gestation by caesarean section. The parents are healthy and consanguineous (first cousins). At the age of 2 months the girl was admitted because of severe feeding problems. Medical examination revealed no malformation of inner organs and a heart murmur turned out to be accidental. Length and weight are within normal range and developmental delay is not obvious until now. Dysmorphic features are flat occiput, hypertelorism, downslanting palpebral fissures, thick eyebrows with medial flare, short nose with depressed nasal root, thin upper lip, preauricular pits, large, dysmorphic ears with prominent antehelix and indentation of earlobes. Frontal hair line is low. Routine cytogenetic analysis revealed a female karyotype with a structural aberrant dicentric chromosome 22. FISH analysis with a chromosome 22 specific painting probe identified the additional material as chromosome 22 specific. The duplicated segment includes the DiGeorge critical region as demonstrated by FISH. The karyotype is described as dup(22)(pter->q12::q10->qter). Chromosome analysis of lymphocytes from the parents revealed normal karyotypes.
Although the girl carries a relatively large duplicated segment the clinical findings are relatively mild. Preauricular pits as observed in the girl are typical for cat eye syndrome. Hypertelorism and downslanting palpebral fissures are also found in 22q11.2 microduplication syndrome. The altered shape of the eyebrows in our patient is similar to the observation in 22q11.2 microduplication syndrome.

P0242. Structural chromatin features in cytogenetic preparations imply genomic separation and epigenetic activities

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Transcriptionally active chromatin is known to adopt a more open structure, for example, through histone acetylation, while deacetylation promotes repression of gene expression by compaction of chromatin [1,2]. Exploration of various gene locii and other markers of human chromosomes following FISH staining revealed a biphasic organisation of nuclear chromatin which may be associated with epigenetic activities. Different sequence and chromosome specific DNA probes, applied singly or combined, indicated that in some cells one of the two haploid sets adopts the open chromatin structure and appears to take the lead to operate, while the other remains quiescent. We have observed this feature during cytogenetic investigations of cells from amniotic fluids, postnatal, juvenile and adult specimens. This feature of apparent functional hemizygosity, besides lending support to the phenomenon of separation of the parental genomes in nuclei all through the cell cycle [3-5], may indeed have implications to epigenetic activities. We are presenting these observations for a general scrutiny because they may be involved in development, and possibly also in cellular differentiation and in neoplastic transformation through loss of heterozygosity [6].

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P0243. Case report: boy with severe congenital heart defect and tetrasomy 9p tissue-specific mosaicism

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Prenatal ultrasound showed a univentricular heart with transposition of the great vessels. Amniocentesis was performed and a normal standard karyotype was obtained. The mother reported no exposure to teratogens and normal fetal movements during the pregnancy. At birth, the mother and father were 22 and 26 years old, respectively. After birth, chromosome analysis was repeated because the infant showed a number of congenital anomalies (bulbous beaked nose, down slanting corners of the mouth, mild micrognathia, and bilateral longitudinal plantar creases) in addition to his heart defect. The analysis of 109 metaphases from blood lymphocytes showed in 15 % of the cells an E-group sized metacentric extrachromosome, which by FISH using CEPH YAC 853b3 was shown to be an isochromosome 9p, in 12.8 % of the cells. A coincidental common 22q11 deletion was excluded. The karyotype was 47,XY,+i(9p)[14].ish i(9p)(D9S1681++) 22q11(HIRA/D22S553/D22S609x2,HCFx2) / 46,XY[95]. In an additional chromosome analysis of skin fibroblasts, the isochromosome was not found in 23 metaphases. This is the lowest level (15% in lymphocytes, 0% in skin) of tetrasomy 9p that has been reported. Congenital cardiac anomalies have been reported in only 1 of 12 (8.3%) patients with trip(9p) mosaicism (Human cytogenetics database, Schinzel 1994), and the type of heart defect observed in our patient has not been reported previously with tetrasomy 9p. The report demonstrates the limitations in the prenatal diagnosis of tissue-specific chromosomal mosaicism. Moreover, it illustrates the clinical variability of the tetrasomy 9p syndrome, which may include univentricular heart.

P0244. Novel exonic point mutations in cystathionine β-synthase gene of Down syndrome

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Recently, folate metabolism has been linked with human aneuploidy such as Down syndrome. Cystathionine β-synthase (CBS) is involved in folate metabolism and the gene is located on chromosome 21. The aim of this study was to detect the presence of CBS gene mutation(s) in Down syndrome children and their mothers using saliva samples. Exon-specific primers were designed from the intron sequences for amplification of the 17 CBS exons. The mutation was detected using the ABI Prism 377 sequencer. Mutations were detected only in exon 10 and 17. A transition of C to T was found at position 20628 of exon 10 while exon 17 has two mutations at position T27796C and C27817T. The Down syndrome children were found to have the same genotype as their mothers. The percentage of the children and mothers having normal, heterozygous, and homozygous transition(s) genotypes were 20%, 40%, 40%, respectively, for exon 10 and 20%, 46.7% and 33.3%, respectively, for exon 17. The number of mothers and children having the transitions in the CBS gene was twice the number of mothers and children with normal genotype, suggesting that the mothers who have these substitutions are at higher risk of having a child with Down syndrome.

P0245. Small reciprocal insertion detected by spectral karyotyping (SKY)

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Insertions are rare, reciprocal insertions most unusual. Reciprocity of insertions has been reported only in three patients. We observed the exchange of small fragments between chromo-somes 4 and 16. A now 6 year-old boy presented with insufficient opposition of the hypoplastic thumbs, thenar hypoplasia, brachydactyly, single palmar creases, cryptorchidism, epicanthal folds, an enlarge-ment of the cavum septum pellucidum, a spinal dermal sinus (i.e., spina bifida occulta), muscular hypotonia, and borderline mental retardation. Hearing test and ophthalmologic examination were normal. The karyotype was unbalanced, 46,XY,der(16)ins(4;16)(q27q28.2;q 12.1 or q13) pat. In the father a reciprocal insertion, 46,XY,rep ins(4;16) (q27q28.2;q12.1 or q13), was found. The father also had brachydactyly but his thenars and thumbs were normal. He had bilateral hearing loss of about 50% which, however, was attributed to frequent otitis media during childhood.
Discussion: The chromosomal origin of the insertion in chromosome 16 of the propositus and its balanced reciprocity in the father were identified by spectral karyotyping (SKY). Cryptic and half-cryptic interchromosomal insertions in the size range of 1 - 5 Mb can be detected by routine cytogenetics if hybrid-ization techniques such as SKY are implemented. In the individuals presented here, FISH analysis with BAC clones is being applied now to characterize the deletion/insertion borders. If the rearrangement comprises the chromosomal region 16q12.1, Townes-Brocks syndrome is to be diagnosed in the son resulting from haploinsufficiency of SALL1 which is located in this region.

P0246. Identification of five new common Fragile Sites that are conserved between human and mouse

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Common Fragile Sites (cFS) have been shown to be relatively unstable genomic sites that may serve as hot spots for tumor related DNA-breakage.
Here, we compared cFS-expression in Balb/c and C57/BL6 mice (five each) to the expression of human cFSs (ten probands). Balb/c mice showed significantly more cFSs than C57/BL6 animals after treatment with different concentrations of aphidicolin (APC), but a similar spontaneous level of cFS expression. Using statistical calculations that take into account the frequency of cFS expression and the size of the respective chromosomal band we identified non-random 30 cFSs (out of app. 240 cFS) in each mouse strain. Of those, 22 cFSs were found to be expressed in both Balb/c and C57/BL6 mice, whereas each mouse strain also expressed eight strain-specific cFSs.
Furthermore, levels of cFS expression were compared between mouse and human for all evolutionary conserved segments consisting of two or more chromosomal bands. We found a high correlation between the frequencies of cFS expression in homologous bands. This finding suggests that the level of cFS expression is a characteristic feature of chromosomal segments that was conserved during evolution.
In addition, utilizing molecular cytogenetic techniques we have identified five cFSs that span the exact homologous sequences in the mouse and human genome (Fra2D-FRA2G, Fra4C2-FRA9E, Fra6A3.1-FRA7G, Fra6B1-FRA7H and Fra12C1-FRA7K). The fact that the genomes of men and mouse differ in particular in their repetitive DNA sequences suggests that cFS expression may not be explained by the occurrence of repetitive sequences.

P0247. Mechanisms leading to the female predominance of somatic and germ mosaicism carriers

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Objectives: Comparative analysis of the cytogenetic profile and sex ratio in prenatally and postnatally detected cases of structural chromosome aberrations of postzygotic origin. Analysis of pregnancy outcome in prenatally detected mosaics for normal line/rearrangement (N/rea) cases. Methods: Review of structural autosomal rearrangements of probable post-fertilisation origin of known sex identified from the literature. Transmitting parents and patients with infertility/miscarriages were excluded. Results: (1) A difference was noted in the composition of mosaic N/rea cases between prenatal (N=85) and postnatal ill-defined [N=82, excluding N/r(20)] cases, with the highest proportion of N/del and N/nonRob translocations in the prenatal group, and the highest proportion of N/dup and N/ring in the postnatal group, presumably due to different maternal age composition. (2) There was a female predominance in the cases of N/Rob translocation, Rob translocation fission, mosaicism for more that one Rob translocation, mosaic and nonmosaic homologous balanced and unbalanced acrocentric rearrangements, in both prenatal (21males/31females) and postnatal (76M/101F) cases. (3) There was a male predominance in prenatally detected N/rea (46M/39F) cases and female predominance in postnatally detected ill-defined N/rea (37M/45F) cases. (4) In the prenatal N/rea group, males were more frequently normal (28M/15F), while abnormalities were more common in females (8M/14F), probably due to sex differences in cell proliferation activity. Conclusions: We hypothesise several mechanisms operating at early stages of human development causing female predominance in somatic/gonadal mosaicism carriers: trisomy rescue, instability of pericentromeric regions in female embryos (Kovaleva and Shaffer, 2003; present data), and stronger selection against abnormal cell lines in male embryos.

P0248. Microdeletion Of The Y Chromosome In Klinefelter Syndrome: Case Report

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We report a patient with Klinefelter's Syndrome and with deletion of Yq interval. Our patient was 24 years old with primery infertility. His 3 semen analysis showed azoospermia. Plasme LH and FSH levels were abnormally high and testosterone levels were (below lower limit) reduced. Each of his testis has a volume of 5 cc. Peripheral blood karyotype analysis showed Klinefelter Syndrome (47,XXY) pattern. Polimerase chain reaction amplification of DNA was performed using the fallowing primers; AZFa (sY81, sY82, sY84), AZFb (sY127,

sY142, sY164, RBM1), AZFc (sY145, sY152, sY153, CDY, BPY, DAZ1, DAZ2, DAZ3). Analysis revealed a single deletion of AZFa region (sY84). Deletion of the AZFa region may be an additional factor for absolute azoospermia in men with Klinefelter's Syndrome. To the best of our knowledge this is the first report of a patient with Klinefelter Syndrome and with microdeletion in the AZFa region.

P0249. A new campomelic dysplasia translocation breakpoint maps 400 kb from SOX9

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Campomelic dysplasia (CD) and autosomal XY sex reversal are caused by mutations within the SOX9 gene on 17q or by translocations outside the SOX9 coding region. All CD translocation breakpoints published so far map 50-300 kb upstream of SOX9 except for one with a distance of 890-950 kb, defining an extended control region. Here, we present clinical, cytogenetic, and molecular cytogenetic data for a three years old CD patient with karyotype 46,XY,t(1;17)(q42.1;q24.3). His clinical and radiological features include right cryptorchidism, severe perinealscrotal hypospadias, tracheobronchial dysplasia, flat nasal bridge, high arched palate, hypoplastic scapulae, thoracic scoliosis, and mild tibial bowing. Fluorescence in situ hybridization (FISH) has shown that the 17q breakpoint in this case maps 400 kb proximal to SOX9. Plasmid, cosmid, and BAC clones from the SOX9 region were selected to establish a 550 kb-spanning colour bar code on DNA fiber preparations for high resolution FISH mapping experiments. We are using this FISH assay for (1) the fine mapping of the 17q24.3 breakpoint of this patient on DNA fiber preparations from lymphoblastoid cells and for (2) submicroscopic aberration screening in other CD patients without mutations in the SOX9 coding region.

P0250. Fluorescence *In Situ* Hybridization characterization of apparently balanced translocation reveals cryptic complex chromosomal rearrangements with unexpected level of complexity.

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The great majority of apparently balanced translocations are associated with multiple miscarriages and normal phenotype. Several mechanisms have been proposed to explain how a small percentage of apparently balanced translocations are associated with abnormal phenotypes. One of the proposed mechanisms, that have not been well investigated, is that apparently balanced translocations may host "cryptic" complex chromosomal rearrangements (CCRs). To test this hypothesis, this study investigated 20 non-pre-selected cases with apparently balanced translocations in order to determine the presence of cryptic CCRs. Multiprobe subtelomeric and whole chromosome paint FISH analyses revealed and further characterised three cryptic CCRs. Two out of three CCRs showed an unexpected level of complexity. The results of this study provided evidence that the link between an apparently balanced rearrangement and the appearance of abnormal phenotype, may be partly explained by the presence of cryptic CCRs. The results also suggested that what is reported as apparently balanced translocation by classical cytogenetics, may host cryptic CCRs, which could be more common than initially thought. The use of both of the above-mentioned FISH methodologies was absolutely necessary to detect the CCRs, because they involve not only terminal but also interstitial, as well as very small or large chromosomal segments. These findings emphasize the need to investigate all prenatal and postnatal cases classified as apparently balanced translocations by G-banding, particularly if family member(s) have abnormal phenotype(s). The clinical significance of prenatal and postnatal identification of CCRs is extremely important, as they are associated with multiple miscarriages, mental retardation and malformations.

P0251. New syndrome - chromosome 19q distal deletion syndrome

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There are very few publications about the distal deletion of 19q in patients with MCA/MR. At present, six cases of de novo proximal deletion of 19q with both MCA/MR and Diamond-Blackfan anaemia or MCA/MR have been reported. The first case with a mosaic deletion of the distal third of 19q was published by us (Mikelsaar Ruth et al 2001, JMedGenet).
Now, we have diagnosed the second case of mosaic distal del(19)(q13.3:) from 301 mentally retarded patients studied, giving the incidence about 1 per 150. The proband is a 12-year-old boy with mild mental retardation and hypotonia, agressiveness, speech impairment, slightly upslanted palpebral fissures, high palate, maxillar hypoplasia, anteverted nostrils, skeletal deformities. Cytogenetic studies on cultured lymphocytes using GTG banding and FISH with TelVysion 19q DNA probes (Vysis Inc) showed the deletion of 19q in 13% of metaphases. His karyotype is interpreted as mos 46,XY,del(19)(q13.33:)/46,XY.
The comparison of phenotypes of all these published cases of distal and proximal deletions 19q showed some common features, such as psychomotor/mental retardation and skeletal anomalies. Our two patients have also clinical features that might be more specific to the distal deleted 19q13.33-qter region, such as malocclusion of teeth, high palate, minor anomalies of nose and absence of major congenital anomalies of the internal organs.
In conclusion, the incidence of the del(19)(q13.3:) among mentally retarded patients is higher than observed, showing that many cases might remain undiagnosed. Phenotypic features of patients indicate to the existence of a new syndrome - chromosome 19q distal deletion syndrome.

P0252. Partial trisomy (12)(q24.2qter) due to a paternal t(4;12)(q35;q24.2)

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We report a case of a 29 months old boy with growth retardation, microcephaly, minor facial anomalies, mental retardation, cerebral atrophy, cerebral ventricular dilatation, hypotonia and pes equinovarus. Conventional cytogenetics showed the presence of additional material on the long arm of chromosome 4. Since at the time of diagnosis blood of the parents could not be obtained, 24-colour-FISH (fluorescence in situ hybridization) was performed and it could be demonstrated, that the additional chromosomal material originates from chromosome 12. After revealing this result to the parents their blood could be collected, and, by conventional cytogenetics a balanced chromosome translocation between the long arm of chromosome 4 and the long arm of chromosome 12 was found in the father. Therefore the karyotype in the patient can be described as following: 46,XY,der(4)t(4;12)(q35;q24.2)pat.
We compare the phenotype of our patient with previous reports of patients with partial duplication of the long arm of chromosome 12 and with subtelomeric deletions of the long arm of chromosome 4. Our investigation provides further information for a better clinical delineation of these two chromosomal aberrations.

P0253. Pure proximal monosomy 6q resulting in a distinct phenotype

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We report on the clinical and cytogenetic findings in a girl seen at the age of 13 months and 3 5/12 years. She is the only child of non-consanguineous parents. Pregnancy was complicated by EPH gestosis and caesarean section was performed in the 38th week because of IUGR and CTG-abnormalities. Psychomotoric development was severly retarded. With 13 months the girl was

able to turn. At 3 5/12 years she can sit on her own but is still unable to walk or to stand without help. During the first 2 years of life recurrent episodes of respiratory distress occurred. Moreover, feeding problems required a permanent enteral nutrition via PEG. By now, the girl is still unable to drink. Dysmorphic features include flat midface, hyperteleorism, downslanting palpebral fissures, depressed nasal bridge and a Pierre-Robin-sequence with micro-/ retrognathia requiering surgery. Additionally, she has got an ASD II. Karyotyping revealed a shortening of the long arm of one chromosome 6 (GTG-banding). Whole chromosome painting showed that the derivative chromosome 6 consisted of chromosome 6 material only. By microdissection with subsequent reverse in situ hybridisation, we could identify an interstitial deletion of band q15-q16.1 (46, XX, del(6).rev ish del (6)(q15;q16.1) de novo). Most cases of partial monosomy 6q described before are either located more distally or combined with a partial trisomy of other chromosomes, all resulting in a different phenotype.

P0254. FISH analysis of Y chromosome abnormalities: possibilities and limitations

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Our report presents cytogenetic (GTG banding) and FISH findings from 11 patients with various numerical or structural abnormalities of the chromosome Y. According to the concrete result of the cytogenetic examination DYZ1, DYZ3, SRY and Y-specific whole chromosome painting probes were used for the FISH analysis. Samples from 3 patients were subsequently examined by means of the PCR method focused on Y-specific sequences (SRY, DYZ1, DYZ3, KALY, PABX/PABY).

Table 1: Clinical findings in patients with chromosome Y abnormalities

Phenotype	Number/Percentage of patients
Gonadal dysgenesis	6 / 55%
Turner syndrome features	4 / 36%
Normal male	1 / 9%

The aim of our study is to describe chromosomal aberrations involving the chromosome Y and compare results of the FISH analysis with the clinical, cytogenetic and molecular findings.

Table 2: Overview of cytogenetic and FISH results

Chromosomal abnormality	Number of cases
derivative Y (mostly dicentric)	7
normal Y (in gonadal dysgenesis)	2
translocation t(X;Y)(p22;p11)	1

Dicentric chromosome Y was the most frequent finding. Besides this we describe one case of monocentric derivative Y (involving inverted duplication of Yp) and one case of cryptic XY translocation. In all but two cases we determined the dysmorphic Y in mosaic (mostly with 45,X cells).
FISH is effective method for determination of derivative chromosomes Y and submicroscopic rearrangements involving the SRY region. Phenotypes of all these patients clearly correspond with FISH findings.
In one patient (karyotype 46,XX) with gonadal dysgenesis (not involved in the table 2) molecular analysis revealed presence of the Y-centromeric sequence (DYZ3) while FISH results were negative. This controversial finding indicates certain limitations of the cytogenetic and FISH analysis. Possible explanations are discussed on the poster.
Our study was supported by grants of the Internal Grant Agency (Ministry of Health, Czech Republic) IGA NE 5685-3 and IGA NE 6912-4 and research project of the Charles University No.111300003.

P0255. Molecular cytogenetic mapping of the breakpoints of the constitutional pericentric inversion inv(10)(p11.2q21.2)

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The pericentric inversion of chromosome 10, inv(10)(p11.2q21.2), is one of the most common chromosomal heteromorphisms in humans. The majority of the cases is familial and lacks recombinations in offspring. Most carriers show neither malformations or dysmorphic signs nor any other recurrent clinical phenotype.
We mapped the breakpoints of this chromosomal polymorphism by means of fluorescence-in-situ-hybridization (FISH). Based on the map positions of spanning and flanking BAC clones the breakpoints in 10p11.2 and 10q21.2 were narrowed down to approximately 50kb. FISH suggested the breakpoints of the inv(10)(p11.2q21.2) to be conserved on the molecular cytogenetic level in five unrelated North German and one Russian carrier.
In addition to the common inv(10)(p11.2q21.2), we investigated two carriers of other pericentric inversions of chromosome 10:
A 3-year old boy with retardation of motoric and mental skills, speech delay and dysmorphic features who carried a de novo inv(10)(p15q22.1) and a 51-year old patient with myelofibrosis and inv(10)(p13q22.1). In agreement with the cytogenetic assignment, FISH proved the breakpoints in both cases to differ from those of the inv(10)(p11.2q21.2) polymorphism.
The application of FISH is recommended for prenatal diagnosis of de novo inversions of chromosome 10 and in case parents are not disposable for cytogenetic investigation to discriminate between the stable frequent variant with breakpoints in 10p11.2 and 10q21.2 and other less frequent variants that could be associated with clinical symptoms. Moreover, future molecular studies have to clarify the molecular mechanisms associated with the formation of constitutional inversions 10.
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P0256. Subtelomeric FISH Screening in Mental Retardation: A Survey Report

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FISH screening for subtelomeric chromosomal rearrangements in patients with moderate or severe developmental delay has demonstrated a relatively high yield, detecting chromosomal abnormalities in 6.8% of 840 patients, with variability of frequency in the different surveys.
We report on the results of the diagnostic activity of our laboratory concerning N. 84 patients, all refrred for mental retardation or global developmental delay of mild to severe grade, and judged as mildly to severely dysmorphic by a Clinical Geneticist. All the male patients phenotipically compatible with fragile X syndrome were screened for FRAXA mutation/ premutation and all had a standard normal karyotype performed. All the positive cases for which a normal parent showed the same rearrangement were considered polymorphisms and hence negative at the screening (n6 2q- and n1 10q).
Three de novo cryptic deletions (1p, 9p, 9q), one apparently balanced cryptic reciprocal translocation rcp(4;18) (parents not yet available) and one deletion (20p) at present not yet tested in the family, were identified (5,95%). Of the cryptic rearrangements identified, only the 9q deletion was detectable at high resolution banding.
The phenotypes of the 5 cases can contribute to the knowledge of the telomeric rearrangements associated syndromes.

P0257. Partial trisomy 19q combined with a translocation of the additional material to chromosome 6q detected by quantitative real-time PCR and FISH in a girl with mild facial dysmorphisms, hypotonia and developmental delay

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We report on a 2 7/12-year-old girl who was referred to us because of psychomotor developmental delay. H is the second child of healthy, non-consanguineous parents. Pregnancy and birth were uneventful. Milestones of motor development were achieved delayed: grasping at 6 months, sitting without support at 16 months, crawling at 16 months and free walking at 2 4/12 years of age. Speech development seemed also to be retarded. H spoke about five words and followed simple instructions.
Conventional cytogenetic analysis revealed a numerically and structurally normal female karyotype of 46,XX. By quantitative real-time PCR analysis of all 46 subtelomeric regions a partial trisomy of the subtelomeric region of 19q could be detected. This result was confirmed by FISH-analysis with a subtelomeric probe for 19q. The additional material of chromosome 19q was localised on chromosome 6q. However, a deletion of the subtelomeric region of 6q could not be detected with the subtelomeric probe for 6q. Conventional cytogenetic analysis as well as FISH with subtelomeric probes for 19q and 6q was also carried out in the parents. However, the parents showed normal results.
The detected chromosomal aberration in H probably occurred de novo. It could be assumed that there is also a very small deletion on 6q which cannot be detected by the subtelomeric 6q probe used by FISH nor by the subtelomeric primer sets used by real-time PCR. H's clinical features are very likely to be caused by the partial trisomy 19q and maybe also by an additional partial monosomy 6q.

P0258. Common Fragile Sites, viral integration sites, evolutionary breakpoints and enzymatic DNA-cleavage - what we know about the mechanisms of chromosomal translocations

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Chromosomal aberrations have been identified in virtually all tumors and reflect genomic changes that contribute to the transformation of normal cells into tumor cells. Using screening techniques, such as chromosome banding analysis, comparative genomic hybridization (CGH) and spectral karyotyping (SKY), chromosomal aberrations specific to many different tumors and their respective developmental stages have been detected. Recurrent chromosomal translocations are the primary aberrations found in leukemias, lymphomas and sarcomas. The principal biological mechanisms causing these reciprocal translocations have not yet been identified.
Common Fragile Sites (cFS) have been found to be hot spots of frequent DNA-breakage. They are characterized as chromosomal regions (1 - 9 Mbp in size) showing allelic differences in DNA-replication-timing and increased DNA-flexibility. In order to better understand what may cause the expression of cFSs, we have:
* studied the expression pattern of cFS in 10 normal probands and in two different mouse strains
* identified several new cFSs in human and mouse, their synteny and their colocalization with evolutionary breakpoints
* started to map viral integration sites in close vicinity of cFSs in HPV-transformed keratinocytes
* investigated genomic instability of a tumor-specific translocation breakpoint using high-resolution array-CGH
* analyzed centromeric breakage in two different tissue types (adenocarcinoma vs. squamous cell carcinoma)
* established an in vivo model of the MLL-gene translocation by treating lymphoblastoid cells with etoposide
* and started to investigate the 3D-chromatin-organization of cFS within the nucleus.

P0259. A patient with an interstitial deletion 46,XY,del(18)(q22.2q23) with normal intelligence and height

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The 18q- syndrome is a deletion syndrome with an estimated frequency of 1/40.000 live births. It is characterized by mental retardation, dysmorphic features and growth failure. Most deletions are terminal, and only few interstitial deletions have been published. We report on a male patient with an interstitial 18 q-deletion without mental or growth retardation.
The 19-year old male was admitted for the evaluation of complex-partial epileptic seizures. He had congenital bilateral ear canal atresia. Development had been normal apart from mild speech delay. He attended a school for the deaf. Family history was unremarkable. Clinical examination showed a marfanoid stature, also seen in his father. Head circumference was normal. There were minor facial abnormalites including asymmetric face, high palate, micrognathia and poorly modelled external ears. Except for a motoric polyneuropathia, internal and neurological examination was normal. Ophthalmological examination, echocardiography and abdominal sonography revealed normal results. Neuropsychological tests showed visuomotor skills below average but were otherwise unremarkable. His IQ was in the normal range. MRI scan showed white matter abnormalities and a unilateral temporal lesion. High-resolution chromosome analysis and FISH analysis yielded a pathological male karyotype with an interstitial deletion (18)(q22.2q23).
We conclude that this previously not described interstitial deletion of 18q has breakpoints distal to the genes responsible for microcephaly, mental and growth retardation. Dysmyelination in our patient maybe related to haploinsufficiency of the myelin-basic-protein (MBP) gene located in 18q23 .Other genes in the deleted region must be responsible for atretic ear canals and facial dysmorphies.

P0260. Molecular Cytogenetic analysis in Williams Syndrome

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Willams syndrome is a neurodevelopmental disorder with multisystemic manifestations caused by heterozigosity for partial deletion of chromosome band 7q11.23 with an estimated incidence of 1 in 20 000 live birth which is accompaned with supervascular aortic and hypercalcemia. We were referred 6 cases on the suspicion of this syndrome.
Cytogenetic analysis was perfomed on GTG-banded chromosome s.Prometaphases were analysed using Image analysis system, no karyotype abnormality was found. Fluorescent in situ hybridization (FISH) analysis was performed, using LSI Williams syndrome region probes.
Case 1 was a 1,5-year boy with hypertelorism, palpebral fissures, srabismus, wide unscrewed nostrils, stenosis of pulmonary artery and ventricular septal defect. In this case Wiliams syndrome wasn't confirmed.
Case 2 was a 4-year old girl. She was born in term, weight-3000g . At the delivery polyhydroamnion and large placenta were noted. At the time of phisical examination several dismorphic facial features were noted: flat back of the head, low hear-line on the forehead, hypertelorism, flat nasal bridge, short turn-up nose with the wide unscrewed nostrils, long filter. Hypercalcemia was observed. She had mild language and cognitive delay, coned fingers and clinodactyly. Visceral anomalies included congenital heat defect as a stenosis of pulmonary artery. Williams syndrome was confirmed, with observation only one signal in each cell. This diagnostics was very useful for further prognosis to family.
Molecular cytogenetic analysis is of great importance for confirmation of microdeletion syndromes where conventional cytogenetic study cannot reveal subchromosomal aberrations and it will be perform also for the other referred cases.

P0261. An unbalanced complex rearrangement involving chromosomes 3 and 14 with intra-chromosomal telomeric sequences.

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We report here on clinical, cytogenetic and molecular cytogenetic findings in a girl with a complex chromosomal rearrangement. Clinical features included mental retardation, atrial septal defect, limb defects and dismorphic traits such as high forehead, hypertelorism, low set ears and short neck. Cytogenetic analysis revealed that additional material was present on the q arm of chromosome 14, in mosaic form, in about 85% of the analyzed cells. It could not be resolved by standard or high resolution karyotyping. FISH analysis, using whole chromosome painting probe for chromosome 14, showed that a large extra-chromosomal region was present within the q arm of the derivative chromosome 14. This was identified as a portion of chromosome 3 using the Octoprobe Multiprobe system. The use of subtelomeric probes for chromosomes 3 and 14 unraveled that additional material, derived from both the q arm of chromosome 3 and the q arm of chromosome 14, was fused to the telomeric end of chromosome 14. The derivative chromosome showed in fact two signals for the subtelomeric probe of chromosome 14 with a signal from the subtelomeric probe of the q arm of chromosome 3 in between. Intra-chromosomal telomeric sequences were observed at the fusion point between the original chromosome 14 and the additional material. Parental karyotype was normal further suggesting that the rearrangement originated from a post-zygotic event. Trisomy for both the q end of chromosome 3 and the q end of chromosome 14 possibly explains the phenotype. Molecular mechanisms for this rearrangement will be discussed.

P0262. Cytogenetic effects of the PU-239 in the workers of Nuclear-Chemical Plant

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The frequency and spectrum of chromosome aberrations in peripheral blood lymphocytes were evaluated in 3 groups of workers of the Nuclear Chemical Plant (NCP) who have various internal Pu-239 contents (I − 1.2-11 nCi; II − 13-20 nCi; III −> 25 nCi) and in inhabitants, living in the radiation safe region (control group). In average 300 cells per individual were analyzed. Frequency of aberrant cells in 3 groups of NCP workers and in control was 1.61±0.36, 2.42±0.52, 2.66±0.30, 1.34±0.17 percent respectively. Differences between groups II, III and control group were statistically significant (P<0.05). The analysis of types of chromosome aberrations in workers of all groups of plutonium plant has shown, that damages of chromosomes arise, basically, due to aberrations of chromosomal type (dicentric, ring chromosomes and atypical chromosomes, pair fragments). Differences on frequency of breaks of chromosomal type between the control and group III were statistically significant (P<0.01). In groups II and III increased level of ”rogue cells” and cells with Double minutes chromosomes (DMs) was observed. Differences between groups II and III of NCP workers and control individuals on these parameters were statistically significant (P<0.01). Observed cytogenetic pictures it is possible to explain by action of incorporated plutonium-239 which is a source α-particles. α-particles cause double breaks DNA and initiate formation of aberrations of chromosome type.

P0263. Wolf-Hirschhorn syndrome (WHS): a new mechanism of familial recurrence and further insights into genotype-phenotype correlation

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We report on a family with two brother affected by WHS.
The first patient died at 18 years of age. He had a MCA/MR syndrome fully consistent with a WHS diagnosis. Karyotype was normal and molecular cytogenetic analysis was not performed.
The youngest brother is now 17 years old. He suffers from severe growth retardation, cleft palate, hypospadias, typical facial appearance, severe mental retardation and seizures. Standard chromosomes were apparently normal. FISH analysis, performed when he was 11 years old, confirmed the diagnosis of WHS (46, XY.ish del (4p)(F26-; IS28-; D4S96**-;** D4S43-; D4S182-; D4S180 X 2). The deletion spanned about 3 Mb, including both WHS critical region (WHSCR) and WHS critical region 2 (WHSCR-2).
The recurrence of phenotype between the two brothers led us to further investigations.
A preliminary FISH analysis with the 33c6 probe (D4S43), deleted in the proband, excluded the presence of a cryptic translocation in both parents. Since the mother presented with some phenotypic abnormalities (facial dysmorphisms, mild mental impairment, febrile convulsions), a skin biopsy was performed in order to rule out mosaicism for the microdeletion. In this occasion a non mosaic smaller deletion was detected, spanning about 1.5 Mb from D4S96 to the telomere**.** Her karyotype was 46, XX.ish del(4p)(F26-; IS28-; D4S96-; FGFR3 X 2; D4S43 X 2). Both WHSCR and WHSCR-2 were fully preserved.
We describe the clinical phenotype of the mother in relationship to the small deletion and discuss this unusual potential mechanism of recurrence of microdeletion syndromes.

P0264. Partial sacral agenesis and short medullary cord in a patient with 18p- syndrome: a rarely reported malformation complex

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The 18p- syndrome is one of the most frequent and well-characterized deletion syndrome involving a segment close to a telomere. Its phenotype is not evidently pathognomonic and the most important associated malformation is the holoprosencephaly spectrum. Partial sacral agenesis has been reported only once (Anderson-Shotwell et al. J Med Genet 1989; 26:70-71).
We describe a 9-month-old female patient referred for failure to thrive starting after weaning at the age of 5 months. She was born by C-section at term with normal birth parameters. Examination showed an alert child with weight <P3, length at P3, and OFC at P10. The phenotype was mainly characterized by marked brachy-/plagiocephaly, prominent forehead, bitemporal hollowing and puffiness over the dorsum of hands and feet.
Blood karyotype was 45,XX,-13,-18,+der(t)(13;18)(q10;q10) de novo, resulting in partial monosomy of 18p.
Xray and MRI surveys revealed partial sacral agenesis with normal S1, small S2, posteriorly displaced and abnormally shaped S3 vertebrae, and the medullary cone ending at D12. Further investigations did not show evidence so far for neurogenic bladder or intestine.
In addition, it was found that the girl had at the age of 1 year complete growth hormone and severe IgA deficiencies, as already frequently reported in 18p- patients.
We conclude that it would be useful to screen persons with 18p-syndrome in order to determine the real prevalence of sacral and/or cord anomalies in this chromosome imbalance.

P0265. Clinical and chromosome breakage Investigation in some Iranian patients referred for aplastic anemia including Fanconi 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). Since the definite diagnosis of FA among AA cases is very Important for patients' management, a total of 25 referred cases for AA were investigated clinically and cytogenetically. In this study the lymphocytes of the patients and their normal sex-matched controls has been treated with mitomycin C alkylating agent. The findings are presented and compared with other similar studies

P0266. Application of chromosome microsurgery in producing FISH probes

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One of the advanced technologies in molecular cytogenetics is chromosome microsurgery. With the help of this technology, molecular genetics and cytogenetics come together in order to get more information about chromosome or part of a chromosome.
In chromosome microsurgery the chromosome of interest or chromosomal region of interest is dissected using a fine glass microneedle under an inverted microscope. The chromosomal DNA isolated by chromosome microsurgery is then amplified employing one of the molecular methods such as PCR. The PCR amplified chromosomal DNA can be used in different applications for example fluorescent in situ hybridization (FISH).
In FISH, fluorescently labelled nucleic acid molecules are deposited in chromatin at the site of specific DNA sequences. By this method unique sequence, chromosomal subregions, or entire genomes can be specifically highlighted in metaphase or interphase cells. The technique is simple in principle. Specific DNA or RNA sequences are first labelled with nonradioactive molecules, for example biotin. The probe and the target chromosomes or nucleic acids are denatured. Complementary sequences in the probe and target are then allowed to anneal. After washing and incubation in fluorescently labelled affinity reagents, a discrete fluorescent signal is visible at the site of probe hybridisation FISH has several applications both in research and diagnosis areas.
In this presentation, briefly, the way to make FISH probes by employing chromosome microsurgery will be explained and some of the applications of FISH probes will be mentioned.

P0267. An Unusual Mosaic Form [45,XO / 46,XX / 46,X,i(Xq) / 47,X,i(Xq),i(Xq) / 48,XX,i(Xq),i(Xq)] Of Turner Syndrome

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Turner syndrome is typical for a 45,X karyotype. It is relatively rare among live births, but common in spontaneous abortion. Chromosomal mosaicism placental mosaicisim is the reason of survival at term and displays the characteristics of Turner syndrome (TS). A female patient with Turner syndrome , 19 yers of age was referred to us. Chromosome analysis was carried out on the chromosome spreads of lymphocyte cultures. One hundred metaphases examined by conventional GTG banding technique. The patient exhibited a mosaic kayotype. She had a classical 45,XO karyotype, normal 46,XX female karyotype, isochromosome 46,X,i(Xq) / 47,X,i(Xq),i(Xq) and 48,XX,i(Xq),i(Xq) mosaicisim. The main phenotype of patient was short stature, web-neck, low posterior hair line, gonadal dysgenesis, mental retardation, primary and secondary amenorrhea. The formation of isochromosome of Xq may be a sporadic event, equally likely to occur on the maternally or paternally derived X-chromosome and that the mechanism of formation may be independent of parental origin. Unusual one X, two X isochromosomes giving rise to 45,XO / 46,XX / 46,X, i(Xq) / 47,X,i(Xq),i(Xq) / 48,XX,i(Xq),i(Xq) genotype brings an attention that can not be explained simply by a sporadic occurance. Also, Turner syndrome who receive the X chromosome from their father have higlier verbal IQ scores and better social cognition than those who receive from their mother suggesting male-female differences in social cognition and verbal intelligence. She had more severe mental retardation than other patients with Turner syndrome referred to our laboratory. GTG banding of her mother did not reveal any mosaic karyotype.

P0268. Turner girls with spontaneous puberty should regularly be checked for XX cell line

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Spontaneous puberty occurs in 5-20% of Turner girls. Data from the literature point that a presence of 46,XX cell line is important for development of spontaneous puberty.
We analyzed 76 girls with Turner syndrome followed until 16 years of age; 28 (36.8%) had X monosomy, 21(27.6%) had in addition cell line/s with no structural abnormalities of chromosome X, 13 (17.2%) had structural changes of X in the second cell line, and 14 (18.4%) had only structural changes of X chromosome. Eleven girls (14.5%) developed spontaneous puberty at an age of 12.8 $\pm$ 1.5 years. The karyotype from peripheral blood lymphocytes in this group was : 45, X0 in 3 (27.2%), 45, X0/46,XX/47,XXX, in 4(36,4%), 45,X0/46, XiXq in 4 (36,4%), and 45,X0/46,X,Xr in 1(9%). Menstrual cycling appeared in four girls. Comparison of the karyotype with those without puberty shows higher percentage of karyotype with additional X chromosome and isochromosome X as a second cell line.
One girl developed endometrial carcinoma. FISH performed on the ovarian tissue confirmed 67% and 90% of 46,XX cells respectively, although 45,X0/47,XXX was her karyotype form the blood.
Presence of 46,XX cell in the ovaries might be crucial for the development of spontaneous puberty. Presence of 46,XX cell line in some other tissues (buccal smear, hair root or fibroblasts) might point to mosaicims in the ovaries. Searching for XX cell line with FISH in different accessible tissues in Turner girls should be mandatory in order to develop appropriate approach towards the induction or maintenance of menstrual cycling.

P0269. WAGR syndrome in a patient with 11p13 deletion due to a de novo unbalanced translocation 5p;11p.

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WAGR syndrome, characterized by predisposition to Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation, is one of the most extensively studied "contiguous gene syndromes". Patients invariably have a germline chromosomal deletion at 11p13 of variable size and nature but always affecting WT1 and PAX6 genes, both located on band 11p13.
Here we present a 37-years-old woman with WAGR syndrome .The patient was born at term after an uncomplicated pregnancy, to healthy unrelated parents. At birth low birth weight and buphtalmo were noted. At 4 months of age ophtalmological examination revealed bilateral subtotal aniridia and ectopia lentis. Subsequently she suffered from numerous respiratory infections, one of them requiring hospitalisation for cardiovascular arrest. At 18 months of age she underwent surgery and chemotherapy for Wilms' tumor. Intestinal malrotation was also observed.
At our evaluation the patient shows disproportionate short stature (<3°p), obesity, OFD at 50°p, dysmorphisms, moderate mental retardation with compulsive behaviour.
The standard cytogenetic analysis showed an apparent chromosome 5p deletion. High resolution karyotyping, indicated by WAGR clinical diagnosis, demonstrated an 11(p12-14.2) deletion due to a de novo unbalanced translocation t(5p;11p). The cytogenetic finding was confirmed by FISH with subtelomeric probes and chromosomes 5p and 11p paintings.
The presented case is another example of reciprocal translocation involving the 11p13 region, infrequently reported in the literature, and producing loss of genetic material at the breakpoints responsible of the clinical picture and probably also of the intestinal malrotation not yet observed in WAGR syndrome's cases.

P0270. Are all cases of Kabuki syndrome due to a 8p22-8p23.1 duplication?

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Kabuki syndrome (KS) is a rare multiple congenital anomaly/mental retardation syndrome with an estimated frequency of 1/32,000 in Japan. It is characterized by postnatal growth retardation, distinctive facial features, dermatoglyphic anomalies, skeletal dysplasia and mental retardation. A broad spectrum of additional features are observed in KS patients such as cardiac and kidney malformations, autoimmune thrombopenia and anemia, growth hormone deficiency and premature thelarche. Molecular basis of KS is unknown. Recently, Milunsky and Huang reported six unrelated patients with KS and a 8p22-8p23.1 duplication found using comparative genomic hybridization and confirmed by BAC-FISH. We collected a series of patients with clinically diagnosed KS. No duplication was detected using high resolution chromosome banding. We are currently studying these patients by FISH using the clones described by Milunsky and Huang. To date 15 patients have been studied, and no duplication 8p22-8p23.1 was detected. Our preliminary results do not confirm the previously described association between KS and 8p22-8p23.1 duplication.

P0271. Detection of *De Novo* Partial Trisomy 22qter

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Characterization of a small chromosomal segment by means of conventional cytogenetics, even when high resolution banding is applied, can be inconclusive.
The identification of the origin of duplicated chromosomal material on 22p, otherwise very difficult to characterize, is offered as an example. It was done using the FISH.
The analysis of peripheral lymphocytes from the propositus by means of conventional cytogenetics with standard resolution, was normal. The FISH with cosmid N85A3, which normally hybridizes to the terminal region of the chromosome 22 long arm, revealed 3 signals, two of which corresponded to the normal position, and one was found on the short arm of the derivate chromosome 22. The causes of the distal chromosome segment duplication often lead to the familial pericentric inversion, parents with the same probe having normal signals on the terminal position of the chromosome 22 long arm. Segmental aneusomy was not detected at original cytogenetic diagnosis because the extra material on the chromosome 22 short arm was compatible with polymorphism of the satellite region of 22p. Clinical features of this 3-year-old boy with a rare *de novo* chromosomal aberration was growth retardation at birth, mental and developmental retardation, including a smaller mandible, genital hypospadia, testicular retention, ventricular and pulmonary stenosis. The authors suggest a possible association of the patient's clinical features with the duplicated material at the distal part of chromosome 22 long arm.

P0272. Human congenital anomalies: Cytogenetic analysis in Jammu and Kashmir, India: Four year study

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A total of 250 children in different age group, suspected to have chromosomal abnormalities were enrolled for chromosome study from 1998-2002. Majority of these children were clinically diagnosed as Down Syndrome while the remaining were suspected to have either an autosomal or sex- chromosomal abnormality other than Down syndrome. Chromosomal abnormalities were detected in 79% cases and in the remaining 21% no chromosomal abnormality was seen. In these 79% cases, Trisomy 21 (43%), either typical or mosaicism was recorded, thus confirming the clinical diagnosis. These genetically confirmed Down syndrome were born to mother in different age group, however, maternal age in most of the cases was below 30 year and the child was either Ist or 2nd issue. To all these parents pre-natal diagnosis was advised. All the 43% genetically confirmed Down Syndromes have been referred to the Rehabilitation center for further management.
Following Trisomy 21,Trisomy 13 has been found the next common autosomal abnormality (1%). No other autosomal abnormality was recorded in the referred patients.
In the remaining 35% patients, Sex- Chromosomal abnormalities affecting only the X-Chromosome was recorded. 17% female had XO/XX mosaicism, and 3% had XO condition. 13% males had XXY constitution and the remaining 2% had XY/XXY constitution. Only one male child was found possessing 49,XXXYY Sex Chromosome constitution.
Present study has helped the clinicians of Jammu and Kashmir in getting the proper diagnosis of their patients. The diagnosis has been found useful in the Rehabilitation Programme of those who had Chromosomal abnormality.

P0273. Cytogenetic Analysis of Mental Retardation in and around Coimbatiore, Tamilnadu, India

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Mental Retardation is not a diagnosis in itself but only a symptom of some underlying disorder. Diagnosis evaluation of etiology of mental retardation is essential in the management of parents as well as for genetic counseling of the parents. Mental retardation can occur in many families from a variety of causes, many of them still unidentified not well understood. Some forms occur more frequently under specific social and environmental conditions. A few of them have genetic origin of biochemical disorders, where the dominant characters seem to occur in particular family groups. Other types are the results of birth accidents or diseases. Mental disability may vary from the borderline of sufficiency to a preformed degree of impairment. The concept of mental retardation engulfs many factors such as social, behavioral, development, etiological, morphological and prognostic implication. Role of genetics as an etiological factor in mental retardation has been gaining more ground of late. Clinical Cytogenetics has provided the importance for the detection of an increasing number of recognizable syndromes associated with chromosomal anomalies. Of the 565 subjects analyzed chromosomal aberrations were observed in 362 subjects. Among the male subjects minor chromosomal aberrations were observed in 134 of 255 subjects and major chromosomal aberrations were observed in 99 of 255 subjects. Among the107 female subjects minor chromosomal aberrations were observed in 64 subjects and major chromosomal aberrations were observed in 65 subjects.

P0274. Cytogenetic Findings in Cancerous and Non-Cancerous Lesions of the Digestive System

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Chromosome instability provides a predisposing background to malignancy, contributing to the crucial genetic changes in multistep carcinogenesis. It is generally accepted that cancer is a genetic disease resulting from multiple genome rearrangements. The Gastrointestinal Tract (GIT) is a frequent site for the development of cancers. A variety of chromosomal aberrations have been identified in various cancerous and non-cancerous lesions of the gastrointestinal tract. Cytogenetic analysis revealed various chromosomal aberrations and rearrangements in 19 of the 60 subjects with non-cancerous lesions and 26 of the 80 subjects with cancerous lesions of the digestive system. The percentage of aberrations accounted to a higher score in the group with cancerous lesions than the group with non-cancerous lesions. Certain aberrations observed in cancerous lesions were also observed in non-cancerous lesions suggesting a predisposing or precancerous condition.

P0275. Refining breakpoints using FISH probes in a set of Turner patients with mosaic karyotypes 45,X/46,X,idic(Xq).

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Clinical presentations in patients with mosaicism for a structurally abnormal Y chromosome, can range from a classical Turner phenotype, through mixed gonadal dysgenesis to phenotypically normal males. Isodicentric Y chromosomes are usually observed to be present along with a 45,X cell line as dicentric chromosomes are inherently unstable and may be lost during mitosis resulting in mosaicism. The variability in sexual phenotype is thought to be related to the tissue distribution and relative proportions of Y material in the developing gonads of the respective cell lines, particularly those with functional copies of *SRY*. However, a further factor which might also have an effect on the phenotype is when the breakpoint in the Yq long arm, results in the duplication or absence of the regions bearing genes for azoospermia *AZFa, b* and *c*. As formation of most isodicentric Y chromosomes involves a break and duplication of either the short or long arms, we have used a panel of FISH probes to determine whether there are any common sequences in Yq which are predisposed to breakage in a series of seven patients with idic(Y)(q11.23) chromosomes.

P0276. Cytogenetic analysis in infertility and birth defects: an Indian Perspective

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Cytogenetic diagnosis was carried out in 6474 patients, presented with birth defects, pubertal delay and infertility in children, adolescents and adults respectively. Conventional G-banding was performed in peripheral blood following standard technique. On an average 50 metaphases were examined for each patient; thus a total of ~323700 metaphases and 51792 karyograms were evaluated using IKAROS imaging software. Chromosomal abnormality was recorded as 50%, 25%, and 5% in children, adolescents and adults respectively, whereby 14% of the total patients were detected with some structural or numerical aberrations. Both structural and numerical aberrations were recorded in autosomes and sex chromosomes, with a higher frequency in former group of chomosomes. Clinical correlation of cytogenetic findings revealed consistent or contradictory pattern of chromosomal configuration. Incident of normal karyotypes in many cases, however, pose urge for employment of FISH, CGH or other molecular genetic techniques. In couples having recurrent pregnancy loss, a pericentric inversion in chromosome 9 was the most consistent anomaly observed in 1.8% cases. Since these samples were collected from different states of India, a cross sectional study revealed prevalence of chromosomal disorders in few states, probably due to poor socio-economic condition, awareness of environmental factors, and/or consanguineous marriage. The whole spectrum of aberrations, chromosomal involvement, clinical correlation and state-wise distribution will be presented and discussed. This study indicates significance of chromosomal diagnosis in undiagnosed illness, prevalence of chromosomal aberration in Indian population and impact of gene-environment interaction on human health. The study also establishes conventional G-banding as the cornerstone of clinical cytogenetics.

P0277. Detection of subtelomeric chromosomal rearrangements by FISH analysis in cases with idiopathic mental retardation with dysmorphic features

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Mental retardation (MR) has a 2-3% frequency in general population. One of its main causes is chromosomal rearrangement, which occurs in 40% of the cases with severe MR, and in 5-10% of the cases with mild MR. However, the etiology is not known in 40% of the cases with moderate and severe MR, and in 70% of the cases with mild MR. As the subtelomeric regions of the chromosomes have rich gene content, designation of cryptic chromosomal rearrangements in subtelomeric regions is of great importance etiologically in cases with dysmorphic features with idiopathic MR. Subtelomeric FISH technique is routinely applied lately in cases with dysmorphic features with idiopathic MR. In our study, FISH with specific probes for subtelomeric regions for all chromosomes was applied to 10 cases with idiopathic MR who suit the determined criterias of scoring, and whose karyotypes were detected to be normal by conventional cytogenetic methods. Deletions of cryptic subtelomeric regions in 4 cases were detected as: 46,XX,del(1)(pter), 46,XX,del(4)(pter), 46,XX,del(5)(pter), 46,XX,del(9)(pter). It is emphasized that criterias in choosing the cases were essential, that submicroscopic subtelomeric chromosomal rearrangements has a high frequency of occurrence, and are important in diagnosis and in the counseling to be provided to the family. The clinical features of the cases were discussed and compared with literature.

P0278. Down's syndrome cluster registered in Republic of Belarus as a possible consequence of the Chernobyl accident.

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The prevalence at birth of Down's syndrome (DS) was analyzed in Belarus for the period of 1981 to 2001. No clear-cut long-term effects of radiation exposure of the population due to Chernobyl accident were revealed. The analysis of monthly prevalence showed a DS cluster in January of 1987. We observed 31 DS cases instead of 14 expected. All known modifying factors (contribution of prenatal diagnosis, changes in maternal age distribution and registration completeness) were shown to play negligible role. The time of appearance and the spatial distribution of Down's syndrome children, born in January of 1987, assume an association with the exposure due to the passage of radioactive clouds. Theoretical backgrounds of the revealed cluster were found in the published results of experimental studies. Nevertheless, insufficient dosimetric information, relatively low occurrence of the disorder, lacking information on proband's families (such as migration and data on trisomy origin) as well as contradictory results of epidemiological studies in Europe prevent from unambiguous conclusions and necessitate the performance of further investigations.

P0279. Disruption of the glutamate transporter gene EAAT2 in a boy with mental retardation, epilepsy and t(1;11)(p33;p13) translocation

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The systematic study of patients with disease-associated balanced chromosome rearrangements is a powerful strategy for the isolation of disease genes and for linking phenotype and genotype. In this context we have investigated the chromosomal breakpoints in a boy with severe mental retardation, epilepsy, cerebral cortex atrophy, psychomotor retardation and a de novo balanced translocation t(1;11)(p33;p13). Fluorescence in situ hybridizations FISH using BACs and PACs selected from the candidate regions identified clones from both chromosome 1 and chromosome 11 that span the breakpoints. In silico analysis of the breakpoint regions showed that on chromosome 11, the spanning clone contains part of the excitatory amino acid transporter 2 (SLC1A2) gene. Subsequent Southern blot hybridizations indicated that the SLC1A2 gene is disrupted between exon 9 and exon 10. The SLC1A2 gene product, EAAT2, is primarily responsible for clearance of glutamate from the synaptic cleft, and is therefore an excellent candidate MR gene. Loss of EAAT2 has been previously reported in amyotrophic lateral sclerosis and Alzheimer's disease.

On chromosome 1 the breakpoint-spanning clone contains a small fragment of an uncharacterised gene. Sequence alignments with human and mouse mRNAs and ESTs from GenBank database indicated that the 5´ end of this gene is very likely incomplete. Cloning the DNA fragments that contain the breakpoints, together

with RT-PCR experiments for determining the 5' end of the novel gene, will soon show if this gene is also truncated by the chromosomal rearrangement and if the rearrangement resulted in fusion genes.

P0280. Two cases of Cri Du Chat syndrome in the same family without a familial translocation or inversion.

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The cri du chat syndrome, a chromosomal disorder characterized by a partial deletion of the short arm of chromosome 5, was first described by Lejeune et al in 1963. About 85% of the cases are caused by de novo deletions, the remanining 10-15% of cases are familial with a parental translocation in more than 90% and a para- or pericentric inversion in 5%. Over 80% of deletions are of paternal origin. Although the size of the lost 5p material leading to the cri du chat syndrome varies, all patients appear to have a deletion of 5p15.2.
Here we present two cases with cri du chat syndrome who are second cousins. The first case (DO) was diagnosed in 2000, when he was 2 months old. He had cat-like cry, microcephaly, round face, micrognathia and hypertelorism. The chromosome analysis with GTG banding showed partial 5p deletion distal to 5p14. Karyotypes of the parents were normal. Four years later, the daughter of the paternal uncle of DO's father gave birth to a baby girl. This baby had failure to thrive, feeding difficulties and was refered to our department by the parents of DO because they had noticed the clinical similarities with DO. The chromosome analysis with GTG banding revealed deletion of the short arm of chromosome 5 distal to 5p14. Karyotypes of the parents were normal again. As far as we know, this is the first family in which the cri du chat syndrome recurs without a familial translocation or inversion.

P0281. Interstitial non-reciprocal translocation with an inverted insertion 46,XY,inv ins(2;4)(p23;q28.1q31.1) associated with primary hypogonadism

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Here we report on a 30 years old male patient with suspected diagnosis of Klinefelter syndrome. Clinical and endocrinological examinations revealed a primary hypogonadism. Conventional chromosome analysis on peripheral blood demonstrated a derivative chromosome 2 and 4 with a suspected balanced reciprocal translocation 46,XY,t(2;4)(p23~p24;q28~q31). Karyotyping of the parents showed a normal paternal karyotype (46,XY) while the maternal karyotype presented a pericentric inversion 46,XX,inv(2)(p11.2q13). Subsequently performed chromosome painting with chromosome 2 and 4 specific libraries revealed that only material from chromosome 4 was translocated interstitially to chromosome 2, hence this process was not reciprocal. To exclude cryptic reciprocity, for evaluation of orientation of the insertion and to enable precise breakpoint analysis, multicolor banding (MCB) was applied. With these results it was proven that (1) neither loss nor gain of chromosomal material occurred with respect to the affected chromosomes, (2) the translocated segment was inverted and (3) that the translocation was a non-reciprocal event. The breakpoint regions at chromosome 2 and 4 harbor a number of genes which can be categorized as candidate genes. One of them, the *steroid-5-alpha-reductase*-gene (SRD5A2; OMIM264600), is located in the breakpoint region at chromosome 2 and is coding for an enzyme which plays a pivotal role in steroid metabolism. Further analyses are ongoing to study whether the patient's phenotype is caused by alteration(s) of this or other genes in the breakpoint regions. Based on former observations of interchromosomal effects we discuss a possible functional association between the translocation identified in the patient and the maternal pericentric inversion.

P0282. Topology of the imprinted Prader-Willi/ Angelman and Beckwith-Wiedemann Syndrome loci in cycling cells

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Spatial association of oppositely imprinted regions in late S-phase but not at other stages of the cell cycle was reported for the Prader-Willi/ Angelman Syndrome (PWS/AS) in human cells (LaSalle and Lalande. Science 272: 725-728 (1996)). We re-investigated this "kissing phenomenon" using 3D-FISH with locus specific probes and replication labeling for a precise S-phase staging. Average relative distances (ARDs) between the two PWS/AS loci were determined in 3D image stacks collected with a confocal microscope. In PHA-stimulated lymphocytes ARD was significantly smaller in late S-phase compared to early S-phase and quiescent lymphocytes. A corresponding decrease was found for chromosome 15 centromeres, which is located in close vicinity to both ribosomal genes on 15p and the PWS/AS region on 15q11-13. In lymphoblastoid cells of a PWS patient with a complete deletion of the PWS/AS region in 15pat we noted the same decrease of ARD in late S-phase. In gorilla lymphoblastoid cells, however, where the human #15 homolog bears the PWS/AS locus but no ribosomal genes, the ARD between the two loci remained unchanged during S-phase. Significant ARD changes during S-phase of lymphoblasts were also not detected for another imprinted locus, i.e. the Beckwith-Wiedemann region on 11q15.5. In summary, our data provide strong evidence against the postulated "kissing phenomenon".

P0283. Genetic investigation in male infertility

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Background. Infertility affects about 15% of couples and approximately in a half of cases males are affected. Among the latter one of the most important cause is abnormal spermatogenesis due to chromosomal aberrations.
Methods. We report the results of the cytogenetic (GTG) and molecular cytogenetic (FISH) study carried out in 68 infertile males with azoospermia as an only selection criterion. Clinical examination and semen analysis were performed according to the WHO recommendations. Cytogenetic and FISH analysis were performed with Axioplan-2mot microscope (Carl Zeiss) and ikaros and isis software (Metasystems).
Results. Cytogenetic abnormalities were 47,XXY karyotype in 6 patients, 46,XY/47,XXY with low level mosaicism in 1 patient, 46,XY,del(Y)(q11q23) in 1 patient, 47, XYY in 1 patient and one 46,XX, male. Thus, 14,7% of azoospermics presented chromosomes aberrations and sex chromosomes abnormalities were in 100% cases. Moreover, it were revealed heterochromatin variants totally in 20,6%: 46,XYqh- in 11 azoospermic males, 46,XYqh+ in 1 patient and 46,XY,inv(9)(p11q13) in 2 patients.
Conclusion. Genetic study is very important for detection of possible causes of male infertility. The received results allow us to suggest the possible role of pericentric heterochromatin polymorphysm in a parentage of an infertility. The correlation between phenotype, spermatogenic abnormalities and the revealed genetic aberrations will be discussed

P0284. The frequency of chromosome aberrations in somatic cells of children living at iodine endemy territory of Ukraine

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The frequency of chromosome aberrations in peripheral blood lymphocytes of children 12-16 years old lived in iodine endemy territory from Lviv region of Ukraine have been determined with G-banding staining. In individuals without morpho-functional disorders of thyroid gland the mean-group rate of chromosome aberrations (1.03±0.32 per 100 cells) corresponded to the population Ukrainian level. These data served as the indicators of the genome stability owing to the strong compensation of children endocrine system under iodine deficiency and confirmed the relative ecology safety of observed territory. In children with chronic thyreoiditis the increased

mean-group frequency of chromosome aberrations (2.10+0.49 per 100 cells) have been observed. Both chromatid (1.05±0.34) and cromosome (0.94±0.33) deletions predominated among all types of chromosome aberrations. The most of chromosome breakes (68%) in euchromatin regions, especially in the band 5p15, have been localizated. Thus, the influence of thyroid gland pathology at the increased chromosome instability have been registered.

P0285. Delineation of the breakpoint regions in two patients with mental retardation, multiple congenital anomalies, and chromosome rearrangements

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Disease-associated, balanced chromosomal aberrations are powerful tools for gene identification. We report two patients with a chromosome rearrangement and a unique disease phenotype. The first patient is a female with microcephaly, Dandy-Walker malformation, aplasia of the vermis, conductive hearing impairment, and severe psychomotor retardation. She carries a paracentric inversion on one of her X chromosomes, 46,X,inv(X)(p11.4;p2 2.32). We delineated the breakpoint regions by fluorescence in situ hybridization (FISH) and identified four BAC clones spanning the breakpoint in Xp22.32 and five spanning that in Xp11.4. The breakpoint in Xp22.32 is proximal to the pseudoautosomal boundary, in a gene-poor region. In Xp11.4, we mapped the breakpoint in intron 5 of the *CASK* gene. *CASK* encodes a protein with similarity to Ca^{2+}/calmodulin-dependent protein kinase II and membrane-associated guanylate kinase. Male mice carrying a transgene insertion in *Cask* show hypoplasia of the mandible and a cleft secondary palate suggesting that *Cask* is involved in craniofacial development.
The second patient is a male with facial dysmorphisms, sensorineural hearing loss, and mental retardation. Cytogenetic analysis revealed a *de novo* 1;10 translocation, t(1;10)(p13.1;p13). By FISH, we narrowed down the breakpoint regions and identified an overlapping clone for each breakpoint. The insert of RP11-188D8 (1p12) contains a large part of the *MAN1A2* gene suggesting that this gene might be disrupted. *MAN1A2* encodes an alpha-mannosidase that plays an essential role in the elaboration of complex and hybrid N-glycans in mammalian cells. Studies are in progress to establish the association between the disrupted genes and the phenotype of the patients.

P0286. Unusual tetrasomy 9p mosaicism in a non-mentally retarded 52-year-old woman with clinical manifestations of scleroderma

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Tetrasomy 9p is a chromosomal aberration which has been described in mosaic status in a few patients. All display abnormal phenotype including variable dysmorphic features and development delay. In the reported case, blood tetrasomy 9p mosaicism was found in a non-mentally retarded 52 year-old-woman . Medical history has included lymphoedema of lower limbs since birth, Raynaud syndrome since childhood, pregnancy-induced-hypertension followed by asymmetrical essential paroxysmal hypertension. Given this symptomatology and , at present, arthralgia of hands, elbows and knees, fat fingers, thick skin, right upper limb oedema and morning stiffness, scleroderma at the beginning has been hypothesized. Morphological examination showed hypopigmentated skin areas on the back and left upper and lower limbs.
A karyotype was performed because of the four pregnancies of this woman, two resulted in late fetal losses and one in unexplained neonatal death; only one pregnancy resulted in a healthy girl. An extra isodicentric 9p chromosome was present in blood cells at a high level (24%) but not in 100% skin fibroblasts, accounting for blood-limited mosaicism. No increased chromosomal breakage was observed. Parental karyotypes of the patient were normal and their medical history was not contributive. Molecular studies demonstrated the maternal meiotic origin of the extra 9p.
This tetrasomy 9p mosaicism report is remarkable for two reasons: first, no development delay is observed despite a high level blood mosaicism; second, this tetrasomy 9p is associated with clinical manifestations of scleroderma. These two unusual aspects of tetrasomy 9p are discussed.

P0287. Identification of repeated sequences at chromosomal breakpoints of translocated Cri du Chat Syndrome patients.

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In recent years, several studies showed that repeated sequences can predispose to homologous unequal recombination, leading both to chromosome microrearrangements (deletions, duplications, inversions) and to supernumerary inverted duplication chromosomes. It seems likely that several chromosome rearrangements may be mediated by the same mechanism. Repeated sequences located on different chromosomes could be responsible also for translocations, as demonstrated for the constitutional t(11;22)(q23;q11).
Cri du Chat Syndrome (CdCS) is associated with a deletion of the short arm of chromosome 5 (5p-), the size of the deletion ranging from the entire short arm to only 5p15.3. CdCS has an estimated incidence of 1:50,000 live births. Most CdCS cases are de novo deletions (90%), parental translocations accounts for approximately 10% and rare chromosomal aberrations about for 1%. Although CdCS is a defined clinical entity individuals with 5p deletion show phenotypic and cytogenetic heterogeneity. At present the etiology of CDC syndrome is unknown and no data is indicating if a gene clusters or specific genomic polymorphisms are involved in mediating the recurrent chromosomal rearrangements present in this syndrome. In this study we examined 3 CDCS patients with an unbalanced translocation involving 5p and another autosome in search of possible repeated sequences at breakpoints that could have been responsible in mediating the translocation events. All 3 patients presented the same breakpoint on 5p, defined by YAC probes 951E9 and 933G1. BAC/PAC contigs, covering the breakpoints on the different chromosomes, were created and their sequences searched for LCRs, LINEs, SINEs with different databases.

P0288. A Romanian population study of 10 Y-chromosome STS loci from infertile men

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About 30% of couple infertilities are of male origin. They appear in some cases de novo and are considered idiopathic. The long arm of the human Y chromosome contains factors which are involved in male fertility. Deletions in AZF region can cause severe spermatogenic defects ranging from non-obstructive azoospermia to oligozoospermia. The intracytoplasmatic sperm injection technique (ICSI) is rapidly becoming a versatile procedure for human assisted reproduction in case of male infertility. But, the use of intracytoplasmatic sperm injection may allow Y chromosome defects to be passed from father to son. The goal of our study is to evaluate the frequency of microdeletions in the long arm of Y chromosome, within the AZF regions, in these cases of Romanian male with infertility. Forty infertile men with azoospermia or oligozoospermia, determined by spermiogram, were studied. Peripheral blood DNA was extracted from each patient, then amplified by multiplex PCR with STS genomic markers from the Y chromosome AZF zones. PCR products were then analysed on agarose gels. Due the difficulty of confirming the absence of a signal in molecular biology, each case was checked by multiplex PCR through coamplification with the SRY marker. Three men with microdeletions of the long arm of the Y chromosome were diagnosed among the 40 patients, corresponding to a proportion of 7,5%.

P0289. Distribution of R- and G-band assigned Bacs in different interphase chromosome territories

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Chromosome territories (CTs) contain G-band and R-band domains. The latter have a higher transcriptional activity and replicate earlier. Using 3D-FISH and confocal microscopy we studied the 3D topology of bacterial artificial chromosomes (BACs) assigned to R- and G-band domains of human CTs #18 and #19. These CTs were chosen, because CTs #18 are built up mostly of G-band domains, while CTs #19 consist mostly of R-band domains. In human fibroblast nuclei we determined (1) BAC signal distributions within each CT, (2) 3D distances of the BAC-signals from the nuclear centre provided by the DNA counterstain, (3) distances of each BAC signal to the corresponding CT border, (4) distances between each BAC signal and the CT intensity gravity centre, and (5) the vertical spacing between G- and R-band specific BACs, respectively. Our analysis did not reveal a significant difference between the 3D arrangements of R- and G-band specific BACs in #18 and #19 CTs. In contrast, previous (Zink et al. Exp Cell Res 247:176 (1999)) and ongoing studies of other CTs, including #12 and #13, indicate a different distribution of R- and G-band specific BACs. Notably, these CTs are much more inhomogeneous with regard to R- and G-band content than #18 and #19 CTs. Whether these differences in band composition cause differences in CT organization remains to be explored.

P0290. Micro-array CGH analysis of 4p microdeletions refines the genotype-phenotype map of the region and pinpoints low copy repeats as susceptibility sites for terminal chromosomal deletions

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The Wolf-Hirschhorn Syndrome is caused by partial deletions of the short arm of chromosome 4 and is phenotypically defined by mental and growth retardation, seizures and peculiar craniofacial manifestations. To improve the phenotypic map of the region and investigate the mechanism by which both terminal and interstitial 4p deletions arise, we wanted to localize the breakpoints of these chromosomal anomalies. To this end, a 4pter specific micro-array was developed consisting of 170 4pter clones and 130 clones derived from the other autosomes and the X chromosome. The complete BAC tiling path of the first 20 Mb and a single clone every Mb between 20 and 47 Mb of the short arm of chromosome 4 was represented. DNA of over 10 patients with aberrations on the 4pter short arm was analysed by array CGH.
Two patients with small interstitial deletions allowed us to further refine the phenotypic map of the region. These analyses pinpoint hemizygosity of WHSC1 as the cause of the typical WHS facial appearance. Our results indicate that the other key features, microcephaly, cleft palate and mental retardation probably result from haploinsufficiency of more than one gene in the region. In addition, our results suggest that a substantial number of breakpoints co-localize with low copy repeats present in the 4p terminal region. This may imply that low copy repeats present in the human genome are not only susceptibility loci for chromosomal rearrangements but, in addition, are susceptibility sites for terminal chromosomal deletions.

P0291. Ring chromosome 15 - Clinical heterogeneity and ring chromosome instability - A case report and Review

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Ring chromosome 15 is not commonly observed, and the clinical manifestations/pathogenesis may be extremely mild and variable. We report here the first case of a ring chromosome 15 among 16,750 cases karyotyped in the heterogeneous population of Kuwait referred for chromosomal analysis.
A young healthy 7 year-old boy with short stature, undescended/retractile testes and mild dysmorphic features was referred for routine cytogenetic investigation. The family history was unremarkable. Proband's 3 brothers and four sisters are all normal. Metaphase chromosomes of proband and his parents were prepared from short term peripheral blood culture and karyotype analysis was done after G-banding, and C-banding techniques by using PS1 power gene karyotyping system.
Analysis of 100 metaphases (G-banding and C-banding) confirmed constitutional 46,XY,r(15) karyotype in majority of all the cells, associated with ring chromosome instability and mosaicism (double ring, dicentric ring, etc.,) Karyotype of the parents were normal 46,XX and 46,XY. confirming *de novo* origin of the ring chromosome formation due to terminal deletion.
Ring chromosome syndrome cases present with extreme clinical heterogeneity and varying degree of prenatal and post-natal growth retardation, and mental retardation with variable severity. Clinical manifestation and pathogenesis in r(15) cases is influenced by the extent of mosaicism and extent of monosomy secondary to the break points. Majority of the cases are sporadic. Formation of a ring in certain cases might be regarded as a „structural mutation", i.e. an alteration in the structure of the genetic material per se, rather than a loss or gain of genetic dosages.

P0292. De novo ring chromosome 6 and eye anomalies

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We report a case of de novo ring chromosome 6 in a 16 week old girl (46,XX,r(6)(p25q27)[100]. She is the second child of normal, healthy parents. Birth weight 1980g, length 41cm, head circumference 30 cm. She remains below the <2.5 centile for weight and length. She is microcephalic and slightly dysmorphic, with a depressed nasal bridge, short palpebral fissures, a deformed and malrotated right ear, a long philtrum and micrognathia. She has dark pigmented spots on both irides and depigmented areas in both retinae. Clinical cardiac examination is normal. Intra-ocular pressure and hearing are normal. Cerebral MR revealed a structurally normal brain. Apart from feeding problems and respiratory infections she is thriving. Metaphase FISH with subtelomeric probes (Vysis) showed no 6p signal but a normal 6q signal in the r(6). Microsatellite marker genotyping and FOXC1 gene analysis as well as detailed cytogenetic findings will be presented.

P0293. Seven fluorochrome multicolor interphase FISH on paraffin embedded tissue sections

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The stronghold of molecular cytogenetics is the analysis of single cells. This is in particular true for interphase FISH, which allows the simultaneous evaluation of various DNA-regions independent of the cell cycle and -if applied to tissue sections- within the natural tissue context. Formalin fixed and paraffin embedded tissue samples represent the vast majority of archival material. If interphase cytogenetics is applied to such archival material it can be corroborated with clinical data to establish the prognostic value of chromosomal copy number changes in a tumor genome. Here, we present advances in technology for the simultaneous analysis of multiple DNA-probes within tissue sections of various thickness. Images are captured using an epifluorescence microscope equipped with a motorized table to collect a stack of images at defined levels in z-direction. For probe labeling we use up to seven different fluorochromes (DEAC, Alexa488, CY3, Texas Red, CY5, CY5.5 and CY7). In addition, DAPI is used for a volume rendering of nuclei which allows us to define the shape and borders of individual nuclei. Subsequently, images are processed with deconvolution-programs to remove out-of-focus information. In a next step probes are color-classified. Finally, color-classified probes and nuclei can be reconstructed to reveal 3D-images. We have developed several multicolor probes which are relevant for different tumor entities. Here we will focus on our current applications on specimen derived from colon tissue. Examples include the evaluation of normal colon tissue and colon adenoma.

P0294. Breakpoint characterisation of two patients with small 14q deletions : genotype-phenotype correlation

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Isolated interstitial deletions of the long arm of chromosome 14 are relatively rare. We describe two patients with small 14q deletions. Patient one is a 3½ year old boy with a 3.5 Mbp interstitial 14q12-q13.1 deletion. Mapping of the deletion was performed using FISH analysis with region-specific BAC clones. BAC clones RP11-26M6 and RP11-81F13 were absent from one chromosome 14 from the patient. Molecular analyses of 16 polymorphic markers helped to narrow down the breakpoints and demonstrated that the derivative chromosome 14 is of maternal origin. In this study, we have also generated a contiguous transcription map of the genomic interval deleted in our patient, linking the regions near markers D14S1060 and D14S286. Several known genes including TITF1, PAX9, and MIPOL1 could be identified in this genomic segment. Patient two, a 11 months old boy, shows a deletion of 14q12-q13.1 between the polymorphic markers D14S262 and D14S975.The deletion was found to be of paternal origin. Some known genes are located within the deleted region including the COCH gene, known to be responsible for autosomal dominant deafness. A genotype - phenotype correlation of this two patients as well as a review of literature was performed.

P0295. A patient with a supernumerary analphoid marker chromosome identified as inv dup 6q associated to terminal deletion 6q.

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We report a child with moderate mental retardation, craniofacial dysmorphism and malformations who was previously found to have a ¨ de novo¨ small supernumerary marker chromosome (SMC_s), from unidentified origin. A re-evaluation at 17 years of age applying spectral karyotyping (SKY) revealed that the marker chromosome was derived from chromosome 6.

FISH analysis. Whole chromosome 6 probe confirmed that marker chromosome was derived from chromosome 6. However, centromeric specific 6 probe (D6Z1) failed to show signal in the marker chromosome, demonstrating absence of α-satellite DNA and suggesting that a **Neocentromere** had been formed. In addition, subtelomeric 6q probe revealed two signals in both terminal ends of the SMC_s and no signal on the distal segment of long arms of one chromosome 6. Therefore, the karyotype was interpreted as a ***inverted duplication of the distal portion of long arms of chromosome 6, associated to a terminal deletion of the long arm of chromosome 6.*** Thus, the patient had a **partial trisomy for the duplicated segment 6q25.3-qter.**

His karyotype was: 47,XY, del(6) (q25.3-qter), + mar. ish del (6) (wpc+, D6Z1+, subtel 6q-), inv dup (6) (wcp+, D6Z1-, subtel 6q ++) de novo.

Microsatellites analysis: Uniparental disomy (UPD) of chromosome 6 was excluded.

Clinical features in our patient are similar to that of other cases previously reported, confirming that the region 6q26-qter is the minimum critical region for the phenotypic characteristics of trisomy 6q syndrome.

P0296. A case of Prader-Willi syndrome caused by an uncommon unbalanced de novo translocation t(15;21) presented with interstitial active NOR and an additional de novo whole-arm translocation t(10;17).

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We report on a 10-year-old male patient with classical features of Prader-Willi syndrome (PWS). High resolution GTG banding revealed a loss of the Prader-Willi critical region (PWCR) originated by an unbalanced translocation t(15;21)(q13;p13) and putative subsequent loss of the derivative chromosome 15. NOR-banding showed an interstitial active NOR in the resulting derivative chromosome der(21). Findings were proved by FISH technique using probes for PWCR (LSI D15Z1,SNRPN,PML). Whole chromosome paints (wcp15, wcp21) and an all telomeric probe confirmed the setting of translocation as described above and could show the presence of an interstitial telomere within chromosome der(21).

Furthermore an apparently balanced whole-arm translocation t(10;17) was detected. Investigations by FISH (wcp10, wcp17; cep10, cep17) and comparative genomic hybridization (CGH) demonstrated the presumed balanced state. Evidently, both translocations, t(15;21) and t(10;17), have arisen de novo - they were not detectable in parental karyotypes. So we deal with an uncommon complex chromosome rearrangement involving four chromosomes causing PWS.

P0297. Constitutional 3p deletion including the entire MITF gene causes an intermediate Tietz / Waardenburg type IIA Syndrome Phenotype

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We report on a 3 years-old patient with symptoms of Tietz- and Waardenburg type IIA syndrome and general developmental delay, psychomotoric retardation as well as mild additonal dysmorphic features. Analysis of G-banded chromosomes of the propositus showed a *de novo* interstitial deletion of the short arm of chromosome 3, del(3)(p14p11). Fine mapping of deletion breakpoints was performed using FISH analysis with region-specific BAC clones. Six BACs (RP11-582M4, RP11-152N21, RP11-450I19, RP11-520D19, RP11-485H21, RP11-544A22) were absent from one chromosome 3. Molecular analyses using polymorphic markers helped to further narrow down the breakpoints and demonstrated that the derivative chromosome 3 is of paternal origin. The deleted segment encompasses about 14 Mb between marker D3S1261 and SHGC-106366. Only a small number of known genes including MITF, which is known to be mutated in the autosomal dominantly inherited Tietz syndrome or in a proportion of Waardenburg type IIA patients, are mapped to this genomic region. This is the first patient with an intermediate Tietz-/Waardenburg type IIA syndrome phenotype that is caused by a constitutional 3p deletion including the entire MITF gene.

P0298. Characterization of six mosaic and non-mosaic marker chromosomes by comparative genomic hybridization

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In the present study we apply comparative genomic hybridization (CGH) in six patients with *de novo* prenatal or postnatal extra marker chromosome, four in a mosaic state and one observed in less than 50 % of cells. In all cases CGH, conventional cytogenetic and FISH techniques allowed the identification of the origin of the extra marker chromosome. After normal CGH results, in two prenatal and one child with an abnormal phenotype (mental retardation, lack of attention, facial dysmorphism, and macrorchidism) heterochromatic composition was deduced. This is supported by high resolution-CGH result and a normal phenotype at birth in one case. In the others three cases, in a boy with facial dysmorphism, hyperactivity and speech delay CGH analyses showed a gain of material in 8p10-p12. According to what was reported our patient showed a mild abnormal phenotype but close to that detected in patients with chromosomal reorganizations of this region. In a case of a healthy man with a history of spontaneous abortions, CGH profile showed a deviation in 8q10-q12. Meiotic study showed an increased of

aneuploid gametes suggesting an interchromosomal effect. In a girl with speech delay, motor skill and object manipulation difficulties, an invdup(15)(q11q13) was characterized. The phenotype is milder than reported manifestations, despite having PW/SA region on the marker chromosome.
We conclude that CGH is a very useful tool to characterize prenatal or postnatal *de novo* marker chromosomes, even when the mosaic state is below 50 % of cells.

P0299. Cell lines of patients with a congenital autosomal-recessive disorder characterised by premature chromosome condensation show in addition delayed chromosome decondensation and a DNA repair defect confined to the condensed chromatin

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Recently, we described a syndrome associated with microcephaly, mental retardation and a cell cycle defect characterised by an increased fraction of prophase-like cells in standard cytogenetic preparations. Cell cycle analysis revealed premature entry of the chromosomes into condensation in the early G2-phase of the cell cycle. Treatment of lymphoblastoid cell lines of these patients with the inhibitor of cytokinesis cytochalasin B six hours prior harvesting, revealed a high fraction of binucleated cells in G1-phase displaying condensed chromatin. We conclude that the cells do not only show premature chromosome condensation, but also a prolonged chromosome decondensation process. Thus, the underlying gene defect is also disturbing timely decondensation in G1 phase after mitosis. These results suggest that chromosome decondensation is a regulated and active process.
48 hours after treatment with DNA-damaging agents or ionising radiation the cell lines of the patients showed no increased sensitivity in flow cytometry studies and chromosome breakage rate was not elevated 24 hours after irradiation. The metaphase index drops drastically already one hour after irradiation, indicating that the induced damage seems to be detected even on the condensed G2 chromatin. However, if cells are exposed to ionising radiation 5 hours before chromosome preparation to increase the fraction of analysed metaphases irradiated in the condensed prophase-like state, a higher rate of chromosome breakage is found in the patient cell lines compared to normal controls. This result points to a possible DNA repair defect which is confined to and specific for the condensed status of the chromatin.

P0300. Trisomy 3q25-qter and monosomy 8p23.1-pter: cytogenetic and molecular analysis with delineation of the phenotype.

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We describe a 4-year-old boy with a karyotype 46,XY,der(8) (8qter→8p23.1::3q25→qter). The dup(3q) syndrome is due to a partial trisomy of (q26.31-27.3) and the phenotype of these patients resembles to Cornelia de Lange syndrome. The clinical findings include typical facial features, mental and growth retardation, genitourinary malformations and congenital heart anomalies. Patients with monosomy 8p exhibit a recognizable syndrome characterized by growth and mental retardation, microcephaly, minor facial anomalies, congenital heart defects and characteristic behavior. Our patient showed delayed mental and growth development, facial anomalies, ventricular septal defect, phimosis, cryptorchidism and behavioral problems. Magnetic resonance image of the brain demonstrated agenesis of corpus callosum and polymicrogiria. The cytogenetic analysis with GTG, CBG and QFQ reveled a 46,XY,add(8p) karyotype. Reverse painting and microsatellite analysis demonstrated partial monosomy of 8p23.1→pter and partial trisomy of 3q25-qter. The data suggest that the chromosomal abnormality was probably originated by a de novo translocation in a paternal germinal cell. We concluded that the phenotype observed in our patient results from the combination of defects described in the isolated dup(3q) and distal del(8p) syndromes and discuss that olfactory receptor (OR) gene clusters may be responsible as a the possible mechanism implicated in this chromosomal rearragement

P0301. Familial acrocentric pericentromeric cryptic translocation 14;22 ascertained by miscarriages

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We describe a family in which several family members had recurrent miscarriages. Routine GTG and RBG banding chromosomes in one family member revealed presence of a *de novo* supernumerary marker chromosome. To characterize this marker we used AcroM-FISH [Langer et al.(2001)HumGenet109:152-158]. As most marker chromosomes are derived from acrocentric chromosomes, AcroM-FISH uses a special probe mix consisting of painting probes for all acrocentric chromosomes, centromere probes for chromosomes 13/21, 14/22, 22, 15, and a probe specific for rDNA, each labeled with a specific combination of fluorochromes. The SMC was stained with the centromere probe for chromosomes 13/21. There was no signal for the rDNA probe or for a painting probe and we described the marker as der(13or21)(pter→q10)(D13/21Z1+). We found in addition a rearranged chromosome 14, where the centromeric region was found to be derived from chromosome 22. Acrocentric pericentromeric abnormalities were recently found in high frequencies in couples with three or more miscarriages [Cockwell et al.(2003)HumGenet112:298-302]. These abnormalities may be the underlying cause of recurrent miscarriages as they may result in abnormal pairing configurations at meiosis. We analyzed the patient's mother, also having a history of recurrent miscarriages. Using AcroM-FISH, we found the same t(14;22). Currently, we analyze other family members, including the grandmother and siblings having no history of recurrent miscarriages. If the t(14;22) segregates together with the history of miscarriages, it could represent further indication that cryptic acrocentric pericentromeric abnormalities may represent a cause for miscarriages. AcroM-FISH appears to be an efficient approach to identify such chromosomal changes and may be included in work-up of couples after miscarriages.

P0302. An apparently balanced de novo translocation t(3;9)(p12-p13;p21-p22.1) in a boy with psychomotor retardation and dysmorphic features

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We report a 2-year-old boy with severe psychomotor delay, muscular hypotonia, and dysmorphic features including prominent forehead with high anterior hairline and small mandible. He is the first child of non-consanguineous parents, born by caesarean section due to CTG worsening at 42nd gestational week of an uncomplicated pregnancy. Birth weight was 2990 g, length 52 cm, and head circumference 36 cm. Statomotor delay was obvious at the age of 10 months for the patient could not turn and crawl. At 22 months of age he was able to walk with holding hands. He did not speak and react to speech, although hearing was normal as was the EEG. Conventional cytogenetic analysis on peripheral lymphocytes revealed an apparently balanced *de novo* translocation, 46,XY,t(3;9)(p12-p13;p21-p22.1). The reciprocal translocation between chromosomes 3 and 9 was confirmed by FISH analysis [t(3;9)(wcp3+,wcp9+;wcp3+,wcp9+)]. Hybridization of a panel of mapped BAC probes to metaphase spreads of the patient delineated the breakpoint regions and mapped one of them proximal to clone RP11-291P10 (3p12) and the second one proximal to clone RP11-220B22 (9p22.1). Further FISH experiments to narrow down the breakpoint regions are in progress. Most of the patients with interstitial deletions including bands 9p21-p13 reported in the literature presented with developmental delay

and minor unspecific anomalies as did our case. Thus, it is tempting to speculate that this chromosomal region harbors a putative gene for mental retardation that is affected in the patient by the rearrangement.

P0303. Analysis of Cell-Free Fetal DNA from Maternal Plasma and Serum using a Conventional Multiplex PCR

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Recent technology enables usage of cell-free fetal DNA in maternal plasma and serum for non-invasive prenatal diagnosis. We compare the success of two different DNA extraction techniques (heat-based or the QIAamp DNA Blood-MiniKit method) on maternal plasma and serum samples in conventional multiplex PCR-setting to determine the best material and protocol to be used for prenatal gender diagnosis. Analysis of material from 5 healthy male and 5 healthy, non-pregnant women revealed gender specificity of either DNA extraction method from either material was 100%, and in multiplex PCR setup the DYS14 sequence represented the male gender, GAPDH sequence was used as an internal control for the presence of extracted genomic DNA. Cytogenetic analysis of cultured amniocytes from 15 pregnant women revealed 11 male and 4 female fetuses. Only PCR results obtained with DNA extracted from maternal plasma using the QIAamp DNA Blood-MiniKit method were in complete concordance with cytogenetic analyses, whereas all other PCR attempts using DNA extracted from maternal serum via either extraction method or from maternal plasma using the heat-based direct method failed to have 100% accuracy for diagnosis of fetal gender. Statistically there was no significant difference between the performance of serum and plasma samples within the same DNA extraction group for either method. The cumulative accuracy of QIAamp DNA Blood-MiniKit extraction (96.6%) was significantly higher than that of heat-based direct extraction (83.3%;$P<0.05$). These results suggest that DNA extraction may be a major success-limiting step and preferably at least one sample from each material, (maternal plasma and serum), has to be utilized for such prenatal genetic diagnosis.

P0303. Partial trisomy 22q11 and tetrasomy22q13 resulting from complex rearrangement of chromosome 22 in a child with distinct morphological phenotype.

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Phenotypic consequences of unusual genome rearrangement of chromosome 22 has been described in a one year old girl seen because of several craniofacial features, developmental delay and agenesis of the left kidney. A detailed catalogue of well-defined traits for systematic morphological descriptions, such as one proposed by Stengel-Rutkowski was used. Chromosome analysis by GTG and RBG showed enlargement short arm of chromosome 22. In M-FISH, the extra material clearly showed the chromosome 22 specific spectral signature. In addition, the Cy3.5 and the Cy5.5 channels are depicted. Chromosome 22 was labeled with these two fluorochromes and the extra material was stained in both color channels, as the normal chromosome 22 material. For further confirmation we hybridized a chromosome 22 specific painting probe, which yielded the same result. In FISH experiments with the commercial DiGeorge probe from Vysis the green probe for 22q13 showed two signals, a small signal close to the centromere and distally a larger second signal un the short arm of the der(22).The red 22q11 probe showed one signal which was flanked by the two green signals. Based on this hybridization we would currently describe the der(22) as der(22)(:22q13->22q?::22q11->22q13::22pter->22qter) and interpretate as partial trisomy 22q11 and tetrasomy22q13 resulting from complex rearrangement of chromosome 22. Further studies are necessary to explain observed rearrangement in details.

P0304. Clinical heterogeneity and Genotype - Karyotype - Phenotype correlations in 18p- Syndrome

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Monosomy for the short arm of chromosome 18 is one of the most frequent autosomal deletions observed in humans . Most cases result from terminal deletion of 18p, while 16% are the result of an unbalanced whole arm translocation resulting in monosomy 18p. The parental origin of these deletions appears to be equally distributed, being half maternally and half paternally derived. From the study of a group of unpublished subjects, observed in our Centre, we report interesting data on the broad clinical spectrum, and on the natural history of this disorder, recently enriched by new aspects.
In particular we report the results of detailed clinical and laboratory studies in few 18p- patients with rare clinical patterns: Turner syndrome- like phenotype, holoprosencephaly - cyclocephaly with proboscis, congenital heart defects, dystonia of the lower extremities and visual defects not accompanied by malformations of the central nervous system, schizophrenia.
Cytogenetic, molecular and Fluorescence in Situ Hybridization analyses of short arm of chromosome 18 were performed on chromosomes and genomic DNA to determine the size and parental origin of the deletion, and to get useful data on the genotype - karyotype - phenotype correlation.
The majority of the breakpoints were located between marker D18s852 on 18p and the centromere, and the distribution of the size of the deletions supports the presence of a breakpoint cluster in the short arm of chromosome 18.

P0305. Genome-wide BAC microarrays: Detection of copy number changes and mapping reduced genomic representations at 1 Mb resolution

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A microarray has been developed to detect genomic copy number changes and map reduced representations of the human genome. The arrays contain 2779 BAC clones with a median spacing of 0.95 Mb. Clone identity was validated by end sequencing, fingerprinting and STS content. Sufficient DNA for printing arrays is assured by generic amplification of BAC miniprep DNA. The clone DNA's and various controls are printed in quadruplicate. Industrial-scale batches of about 80 slides are qualified by SyBr Green staining and hybridisation in real experimental conditions. Signal/noise ratios ranging from 4 to 200 are typical, thus providing a 50-fold dynamic range, with an average signal/noise ratio of about 8 for self-self hybridisations. Detection of copy number changes is illustrated by analysis of DNA isolated from human tumor tissue. The arrays enable the detection of amplified and deleted regions in tumors with turbulent chromosomes, despite the presence of substantial levels of DNA from euploidic cells. Two examples of mapping reduced representations are shown. The arrays are used tomap reduced representations generated by chromatin immunoprecipitation (ChIP-chip). IntegraGen's Genome Hybrid Identity Profiling (GenomeHIP) produces a reduced representation from a mixture of the genomes of affected sib-pairs, such that the final product is enriched for DNA that is "identical by descent" between the siblings. The BAC arrays are used to simultaneously map the GenomeHIP reduced representation and evaluate copy number changes.. Processing of hundreds of arrays of affected sib-pairs has revealed genomic loci, and subsequently genes, linked to complex diseases such as obesity and autism.

P0307. Sex determination by Nested-PCR using amelogenin gene

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Sex determination is to determine fetus sex in X-linked genetic diseases. The aim of this project in clinical aspects is to determine the sex of fetus in initial stages of pregnancy in pre-pregnancy diagnosis as well as IVF cases, biopsy being done from fetus. At first, the system being optimized by blood test on 16 male and female candidates and then 74 sample's chorionic villi (C.V) and sampling was done by transabdominal. Sample's sex being determined by nested-PCR and this technique has shown high specifity and sensitivity. The system sensitivity increased up to amplification of a single cell and it was validated by amplification of DNA from fertile's oocyte. Also, the DNA of 16 samples of human fetus, which were in different cellular stages, and sex of 13 fetus being determined.The target gene in this research is amelogenin which is on X and Y chromosomes thus this system is contained to a positive internal control. After PCR in second round, observation of 484 and 672 bp fragments indicating that its sex is male, and observation of only 484 bp fragment indicating that its sex is female. In the other hand , the observation of 484 bp fragment indicate the effectiveness of the system and 672 bp fragment will determine the sex. In single cell PCR in order to prevent amplification only single allele or preferential amplification will increase the initial temperature of denaturation so that to prevent the occurrence of allele dropout .

P0308. A Novel Microdeletion, Del(2)(q22.3q23.3) in a Mentally Retarded Patient, Detected by Array-based Comparative Genomic Hybridization

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A novel interstitial submicroscopic deletion in the long arm of chromosome 2 detected by genome wide array-based comparative genomic hybridization (arrayCGH), is reported in a mentally retarded 14-years old girl with additional facial dysmorphisms. The deletion included three clones (RP11-89L3, RP11-90K5, RP11-72H3) mapping in a 2 Mb genomic interval located on 2q22.3q23.1, just distal from the Mowat Wilson critical region (SIP-1). Molecular analyses of 11 polymorphic microsatellite markers delineated the deletion to a 3-5 Mb region spanning 2q22.3q23.3 and demonstrated the maternal origin of the derivate chromosome. Similarities between clinical features in our patient and in patients with deletions including the 2q22.3q23.3 region were observed, such as psychomotor retardation, hypotonia, microcephaly and facial dysmorphic features. The deleted region encompassed 9 well transcribed sequences including four known protein coding genes (Activin A Receptor type II gene, the Origin Recognition Complex subunit 4 gene, DKFZP566F2124 and the Kinesin Family Member 5C gene). Haploinsufficiency of one or more genes within the deleted region could be causative for the phenotype. In the near future an increasing number of submicroscopic deletions and duplications will be identified by arrayCGH. Clinical comparison of cases with overlapping deletions will be imperative to understand the meaning of these findings and will allow us to identify genes involved in mental retardation and human malformation.

Prenatal diagnosis

P0309. Actual efficiency of prenatal diagnosis in IInd trimester

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Survey of results of 1000 amniocenteses done between 2001-2003 (two and half year). Detection of inborn chromosomal aberrations was 1.9% (19). Only 8 were M. Down - trisomy 21. One was Edwards syndrome, two were Patau syndromes, one partial deletion of 18q, one partial trisomy of chromosome 5q and the rest were abnormalities of sex chromosomes. Type of indications: 26% age-indications (35 years and more), 36% were abnormal results of biochemical screening (Tripletest -AFP, HCG, E3), and 42% ultrasound abnormalities. Ultrasound examination is now more effective than before. However the age of pregnant women is increasing in our country and therefore age-indications will be more frequent in the future. Generally about 60 % of inborn chromosomal aberrations are detected in Czech Republic by prenatal diagnoses. All pregnancies with pathological karyotypes were terminated in our group under study.

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P0310. Cystic Hygroma: unexpected cytogenetic study

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Although the ultrasonography development allows the early detection of fetal development and since the ultrasound at 10-14 weeks of gestation is a routine analyse to detect congenital anomalies, including the cystic hygroma, we continue to find several situations where a late diagnostic has been done. Unfortunately the Health Promotion and Education is not yet a reality of our country.

We report a case of a young pregnant woman with an abnormal ultrasound scan at 17 weeks of gestation. It revealed an abnormal fetus with several ecographic anomalies: cystic hygroma, anasarca, oligohydramnios, ascytis, pleural effusion, no bladder was found and the umbilical cord had two vessels.

It's a young, health, non-consanguineous couple, with a normal obstetric and familial history (the first pregnancy resulted in a normal boy).

At the prenatal counselling, the couple understood the situation and elected termination of pregnancy and once there was no amniotic liquid, we opted to do the punch in the cystic hygroma for cytogenetics study of the fetus.

After the termination of the pregnancy, blood, skin and placenta, were taken to cytogenetics studies. The blood and cystic hygroma karyotypes were 45,X and 47,XX,+20/46,XX (90-10%) respectively. Unfortunately it was not possible to achieve a result with skin and placenta.

Whenever we have hygromas we attempt to make comparative studies between blood and cystic hygroma`s results. This case was the first that we found a difference between them.

P0311. Quick procedure for quality testing of culture medium in prenatal cell culture

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To increase the reliability of amniotic fluid (AF) cell culture, quality of new batches of medium needs to be evaluated before use. Until now, growth curves of secondary AF cultures were compared for new and old medium over several days. The new batch was only introduced in routine if growth was better or equal. Since this method is not objective, we wanted to develop an absolute test based on number of clones and culture failures in primary AF cultures. Differences in quality might be better detectable under sub-optimal conditions. Therefore, we cultured AF cells in medium containing different proportions of PBS. Primary cultures were started with 0.8 ml pooled AF. Undiluted medium served as control. The number of clones was counted on days 6 and 8. A dilution to 30% appeared to a 50% reduction of number of clones. Three cultures of 10 individual patients were initiated. The average number of clones was 4 (SD 2.93). One culture failed. Based on this result, we set the threshold for the minimum number of successful cultures at 26 out of 30 and the minimum mean number of clones to 3, theoretically leading to a bad result due to random fluctuations only 1 in 100 times. The procedure has been tested with different batches of medium resulting in uniform data. This procedure implies a high probability that no medium will be accepted with less than required quality, while rejection of batches that would have sufficient quality if tested more extensively will be minimal.

P0312. Haplotype Analysis of Related ATM Markers Facilitate Prenatal Diagnosis in Iranian Ataxia Telangiectasia Patients

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Ataxia Telangiectasia is an autosomal recessive disorder in 1/40000 to 1/100000 in reported populations. There is 25% possibility for having an affected child when parents are carrier for ATM gene mutation. There is no cure available for this disease and prenatal testing is strongly recommended in preventation of this disease.Although preference method is the direct mutation analysis of ATM gene, but large size of the ATM gene with 63 exons and the large number of possible mutation in patients considerably limit the facibility of mutations analysis as a choice in diagnosis. Indirect method is a better tool when parent are not carrier of founder mutation and pass different mutations to their children. Indirect molecular diagnosis using ATM related molecular markers facilitate prenatal diagnosis of AT children. In this study four molecular markers: *D11S2179,D11S1787,D11S535,D11S1343* are genotype in 18 unrelated families from different region of IRAN. Those markers are amplified using extracted sequence primers from Gene Bank with their described PCR conditions. The amplified products were separated using denaturing PAGE gels, and the data were analyzed to detect their pattern of inheritance in each family. In all families segregation of alleles were recording to mandelian inheritance and affected chromosomes were distinguishable from unaffected ones. All carriers and affected patients were diagnosed accurately. Thus this method is effectively usable in prenatal diagnosis of ataxia telangiectasia

P0313. Confidential enquiry into pre-natal genetic care

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Introduction: Confidential enquiry is an accepted form of clinical audit that scrutinizes factors which may influence quality and outcome of care. Genetic prenatal diagnosis is part of routine ante-natal care for women with a known a priori risk for a genetic disorder or for those who are identified to be at increased risk by maternal serum screening and/or fetal ultrasound. This study was undertaken to assess how obstetricians manage the genetic aspect of care for these women.

Methods: The study was designed to investigate the antecedents and sequelae of positive cytogenetic and molecular tests and of positive ultrasound findings. A total of 19.334 pregnancies were followed at different health care settings including university hospitals and private practice.

Results: The majority (76%) of women with positive test results were offered qualified pre-test genetic counseling although major differences were observed at different settings ranging from 59%-92% of women who received counseling. However only 29% of all women received qualified post-test counseling – again significant differences (17%-100%) in different settings were observed. Termination of pregnancy took place at an average of 3.8 days after the information of the test result was provided (range: 0-13 days). 86% of all terminations took place until 24th week of gestation (overall range: 11+6 – 32+3 weeks).

Conclusions: Whilst genetic testing is increasingly offered in pre-natal care, only a minority of women undergo qualified genetic counseling after a positive test result.

The study was funded by the German Human Genome Project.

P0314. Unchanged birth prevalence of Down syndrome in Denmark 1969-2002 - effects of prenatal diagnosis and increased mean maternal age

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The Danish Cytogenetic Central Register has a complete registration of all cytogenetic analyses carried out in Denmark since 1969. The first prenatal diagnosis in Denmark for a clinical purpose was carried out 1970, and in 1978 it was decided politically to offer prenatal diagnosis to all women >35 years. Currently around 10% of all pregnancies are investigated for chromosome abnormalities using chorionic villus sampling or amniocentesis.

From theoretical calculations based on the increased risk of Down syndrome associated with maternal age it can expected that prenatal diagnosis in women > 35 years (with subsequent termination of pregnancies with Down syndrome) would lead to a reduction in the birth prevalence of Down syndrome with approx. 30%. During the period 1969-2002 the incidence of children born with Down syndrome fluctuated between 0.66 and 1.17 per thousand liveborns/yr. No significant decline in the incidence was registered over time. In the same period the incidence of prenatally diagnosed fetuses with Down syndrome increased from zero to about 1,1 per thousand liveborns/yr. The main reason for these observations is the increase in mean maternal age (from 24 years to 30 years).

The Danish National Board of Health is about to propose new guidelines for prenatal counselling offering Nuchal Translucency ultrasound screening and/or serum screening to all pregnant women. Data on the past and present developments will be presented.

P0315. Mutation detection and prenatal diagnosis of iranian families with SMA (I-III)

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Spinal muscular atrophy (SMAs) are autosomal recessive disease, in its severe form, after cystic fibrosis, in its milder forms, the second most common pediatric neuromuscular disease after Duchene muscular dystrophy. Depending on the age at the time of onset and severity, SMA is classified into three types (Werdnig-Hoffmann=type I, intermediate form=type II and Kugelberg-welander=type III). SMA causes early death (type I) or increasing disability in childhood (type II, III). All three types of SMA map to chromosome region 5q11.2-q13.3. Homologous deletions in exon 7 and 8 of the survival motor neuron (SMN) gene have been described in >96% of patients with SMA.

The aims of this study were to screen the deletions of SMA gene (exon 7 & 8) and NAIP gene (exon 5 & 6) in Iranian patients for prenatal diagnosis of SMA. We have studied 47 families with SMA types I-III, partly with their affected children and their chorion villus samples (CVS). DNA deletion genotypes were determined by PCR-RFLP analysis amplifying exons 7 and 8 of SMN. Results revealed the homozygous deletions of exon 7 and 8 of the SMN gene in 46/47(97%). 19 samples (CVS) from the pregnant women with affected children, have been tested for deletions of exons 7 and 8 of SMN gene.

The percentage of homozygous deletions in the study is almost as high as that reported in other investigations. This method is useful, fast and effective for gene diagnosis and prenatal diagnosis of SMA.

P0316. Prenatal diagnosis of CATCH22

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Foetal conotruncal malformation was diagnosed during IIIrd level ultrasound indicated by high maternal alpha-fetoprotein in the second trimester. Amniocyte interphase FISH revealed deletion of the DiGeorge syndrome critical region (DGCR). Because of mother's facial dysmorphy, cleft palate and uterus septus the same FISH assay was performed on her peripheral blood with positive result as well. Family decided pregnancy termination. Further genetic analysis revealed double heterozygozity of common methylenenet etrahydrofolate reductase (MTHFR) gene polymorphisms - C677T: CT and A1298C :AC at both parents and foetus as well. CATCH22

inheritance, possible phenotypic effect of MTHFR polymorphisms and our prenatal diagnosis system are discussed.

P0317. Analysis of meiotic segregation products of different kind of balanced chromosomal rearrangements in sperm nuclei of carriers; FISH analysis with different probe combinations

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Balanced chromosomal rearrangements could lead to unbalanced segregation patterns during the meiosis. Fetuses or live born offsprings don't indicate real information about meiosis segregation products due to early missing of abnormal conception. We present our primary findings for evaluation of products of different kind of balanced chromosomal rearrangemets including; Robertsonian and reciprocal translocations, paracentric and pericentric inversions. Semen samples were obtained from these balanced rearrangement carriers. The use of interphase sperm-FISH technique with different probe combination, in sperm nuclei has proved to be an accurated approach to determine products of meiotic segregation and could provide to better establish the reproductive prognosis and genetic counseling. The different kind of centromere, locus and telomere specific probes including rearranged regions of chromosomes were used to compare the frequency of recombinant and nonrecombinant meiotic products. According to our results we conclude that the frequency of sperm nuclei bearing abnormal signals, representing recombinant segregation products of reciprocal translocation carriers was more than other rearrangement carriers and also paracentric inversion carriers had the second frequency of recombinant segregation products. The fertilization of these gammets bearing recombinant chromosomes constitutes an embryo to have trisomic or monosomic for these chromosome regions.

P0318. Ten years of preimplantation genetic diagnosis: outcome of pregnancies and children follow-up

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During the last ten years (1992 till 2002) preimplantation genetic diagnosis (PGD) was offered to 344 couples at risk for monogenic diseases such as cystic fibrosis or myotonic dystrophy or chromosomal aberrations such as robertsonian and reciprocal translocations to avoid affected foetuses or children. Preimplantation genetic diagnosis for aneuploidy screening (PGD-AS) was offered to 353 infertile couples to possibly improve the success rate of their in vitro fertilisation treatment. In the respective groups, 716 cycles led to 125 deliveries and 487 cycles to 73 deliveries. In total 243 children were born of which 74 twins and 12 triplets. Eleven children of which 2 singletons were stillborn or died in the neonatal period. For the singleton children the median gestational age was 39.2 weeks and the median birth weight was 3335 grams with a range between 1400 and 4630 gram. The major malformation rate in the total cohort of children was 2.5%. Compared to children born after in vitro fertilisation (IVF) with or without intracytoplasmic sperm injection (ICSI) the stillborn/neonatal death rate is high but gestational age, birth weight and malformation rate compare favourably. Although the number of PGD children is still small it seems that embryo biopsy is not causing harm to the children of whom more then 50 % are younger than 2 years old

P0319. Outcome of preimplantation genetic diagnosis for myotonic dystrophy

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Preimplantation genetic diagnosis is an alternative to prenatal diagnosis for patients at risk of transmitting an inherited disease to their children. Patients have to go through in vitro fertilization in order to produce embryos from which one or two cells are removed at the 8-cell stage for diagnosis. Since not affected embryos are transferred, PGD avoids the need for the termination of an affected pregnancy. Direct analysis of the CTG triplet was used for fully informative couples and for half informative or not informative couples the Tripled Primed PCR (TP-PCR) protocol was applied. Between 1995 and 2002, 122 PGD cycles were performed in 44 couples. The mean age was 31.5 years. In 11 of the couples the man was affected, in 33 the woman. Reasons to opt for PGD were infertility, objection to termination of pregnancy or bad experience with previous termination.
In 94 of the 122 cycles with oocyte retrieval, at least one unaffected embryo could be transferred to the uterus. Twenty four pregnancies with 31 positive fetal heart beats (18 singletons, 5 twins, 1 triplet) were recorded. After 2 reductions of multiple pregnancies, 2 miscarriages and 1 termination for a misdiagnosis, 21 deliveries occurred and 26 children (16 singletons and 5 twins) were born. With a delivery rate of 17 % per cycle, 22 % per transfer and 48 % per couple (after a mean of 2.8 PGD cycles) PGD seems to be a reasonable option for those couples of which one member is affected with myotonic dystrophy.

P0320. Prenatal cerebral enlarged ventricles, choroid plexus cysts and 22q13 monosomy: part of the syndrome or fortuitous association?

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At the term of 20 weeks of pregnancy, a fetal UV examination showed bilateral cerebral ventriculomegaly (11-12 mm) and choroid plexus cysts. Fetal cerebral MRI showed no associated abnomaly. Fetal karyotype (RHG banding) on amniotic cells was normal : 46, XX. Fetal growth was normal. At birth, the child presented with global hypotonia. Neonatal cerebral MRI showed persistant ventriculomegaly but choroids plexus cysts had receded. At 7 months of age, she presented with severe developmental failure and slight dysmorphic traits. A new karyotype on blood cells with RMBG banding revealed a de novo 22q13 monosomy, confirmed by FISH analysing with the 22q11 probe which showed lack of a control spot in 22q13.
22q13 monosomy syndrome is not characterized by any specific symptoms. Affected children present with severe neonatal hypotonia, developmental failure, and slight dysmorphic traits, without growth failure. Diagnosis of all reported cases of 22q13 monosomy were done on karyotypes with RMBG banding or, by chance, when looking by FISH for the DiGeorges syndrome 22q11 deletion which showed lack of a control spot in 22q13. As there is no specific prenatal sign of this syndrome, no prenatal diagnosis of 22q13 monosomy has been reported. In our case, association of prenatal ventriculomegaly and choroid plexus cysts with 22q13 monosomy could be fortuitous but it raises the question of searching for this deletion in case of prenatal ventriculomegaly leading to a fetal karyotype. It is an easy test to do with the 22q11 probe and 22q13 monosomy is a very severe syndrome.

P0321. Aneuploidy screening in polar bodies from 650 ICSI cycles using FISH

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One out of ten couples worldwide suffers from infertility. Consequently, 1 in 100 newborns in Europe and USA has been conceived following artificial reproductive technologies, most importantly in vitro fertilisation (IVF) and intracytoplasmatic sperm injection (ICSI). However, especially in older women the implantation rate after ICSI is low. In countries where preimplantation diagnosis is not possible by law, five-color FISH on polar bodies can be used to detect aneuploidies for chromosomes 13, 16, 18, 21 and 22 in fertilised eggs. This can be expected to increase the implantation

rate and decrease the number of spontaneous abortions. One major benefit of testing the above chromosomes is prevention of a child with trisomy 13, 18 or 21. We performed first and second polar body diagnoses on fertilised eggs from 500 patients with more than 650 ICSI cycles because of advanced maternal age and/or more than three previously failed cycles. Only embryos showing no detectable aneuploidies for the tested chromosomes were retransfered. The pregnancy rates were 17.0% in woman up to 34 years, 17.8% in women between 35 and 39 years, and 7.7% in women over 39 years. Although the observed low rates may be mainly due to maternal/ parental factors, the commercially available probe set used may not be optimum to predict successful implantation of the tested eggs. Therefore, we have developed an additional probe set for large gene-rich chromosomes for which aneuploidies may already exert negative effects during preimplantation development.

P0322. Hyperechogenic fetal bowel: dilemmas in counselling.

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Dense fetal bowels, detected by ultrasound examination in the second trimester of pregnancy, may be a harmless observation or a sign of a pathologic condition. We report the case of a 24-year-old woman who came for counselling. An amniocentesis was offered. The karyotype of the fetus was normal, however one mutation for Cystic Fibrosis was identified. Dilemmas in counselling this particular case are discussed and follow-up results are presented.

P0323. A case of chromosomal aberration in fetus

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A 26-years old woman was consulted for determination of genetic risk for fetus because of epilepsy and necessity of constant use of phenobarbital and valproat. Her 37-years old husband suffered from mental disease and had some phenotypic microanomalies: dolychocephaly, palpebral fissures slant up, epicanthic folds, high palate, dry skin and café au lait spots. At 12 weeks of pregnancy pathologic meaning of PAPP-A was revealed (0,41 MOM), while AFP and hCG were normal. AFP, hCG, uE_3 determined at 16 weeks were also normal. Sonography conducted at 12 and 22 weeks of pregnancy presented no markers of chromosomal pathology. Taking into consideration abnormal result of PAPP-A and pointed peculiarities of parents prenatal invasive test was offered to the family. The fetus' karyotype turned to be 46,Xr(Y), parents karyotypes were normal. We noted the fact that chromosomal aberration accompanied with the only marker (PAPP-A), while other biochemical and sonography tests were normal.

P0324. Fetal DNA analysis in maternal plasma: new perspectives for noninvasive prenatal diagnosis.

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Recent studies showed the presence of fetal DNA circulating in plasma of pregnant women. This may represent an source of fetal genetic material which can be obtained noninvasively and opens up new prenatal diagnostic applications. Fetal DNA was quantified by SRY real-time PCR in 1583 women with physiological pregnancies from 6 to 40 gestational weeks (median: 8.9, 17.3 and 51.4 ge/mL of maternal plasma in the I, II and III trimester of pregnancy). A subset of 400 samples analyzed blindly displayed 97.3% sensitivity, 100% specificity, 98.6% accuracy in fetal gender determination. We also analyzed 55 multiple pregnancies with at least one male fetus. SRY quantification correlates with the number of male fetuses. For noninvasive testing the complete clearance of fetal DNA after delivery is mandatory. Short-term fetal DNA persistence was detected in plasma of 47/105 women within 2 days after delivery: 12/13 samples re-tested within 3 days scored negative. No long-term persistence was detected in 172 women with previous sons or abortions. SRY quantification in pathological pregnancies revealed high fetal DNA increase in preeclampsia (median 168 ge/mL) and isolated IUGR (median 155 ge/mL). For noninvasive diagnosis of genetic diseases we evaluated a microchip technology. We set up assays to detect 9 beta-thalassemia mutations. The detection limit for a mutated DNA sample diluted into a wild-type DNA sample was 5 ge. Peptide nucleic acids (PNA) addition to the PCR reaction and the microchip allowed 50% inhibition of the wild-type allele signals. Funded by Telethon GGP02015.

P0325. Rapid FISH analysis – experience of over 10,000 samples

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FISH has proved to be a reliable tool for rapid prenatal diagnosis of genetic abnormalities in uncultured amniocytes taken from over 10,000 pregnant women in Germany referred for genetic screening of the fetus. Women were counseled about the different invasive methods of testing. Consenting women had 4–5 ml of amniotic fluid taken for cell culture, and a further 2–5 ml for a FISH assay. Sampling was limited to a maximum of 8 ml in early gestation. FISH testing was done on a minimum of 30 nuclei using the AneuVysion kit (Abbott Diagnostics) with specific probes for chromosomes 13, 18, 21, X and Y. FISH was successfully performed in over 95% of cases and showed 99.3% concordance with karyotype analysis in diagnosing normal cases, 0.7% were suspected to be mosaic (Table1). Of 424 samples found to be aberrant by karyotyping, 338 were found to be aberrant also by FISH, 35 were suspected to be mosaic and 51 were found to be normal by FISH. Over half of the aberrant karyotypes deemed normal by FISH had no phenotypic relevance (eg. balanced translocations, inversions). There was only one false-negative finding (a 45,X case- 0.01%). With FISH 64 cases were classified as suspected mosaics. Most of these were found to be mosaics by karyotyping and a small number were non-mosaic aneuploids. There were no false-positive results.

P0326. Prenatal diagnosis of mosaic sex chromosome aneuploidy.

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Prenatal diagnosis of sex chromosome mosaicism represents serious genetic counselling problems due to possible abnormalities of sexual differentiation. We report a prenatally diagnosed sex chromosome mosaicism 47,XYY/45,X/46,XY and the outcome of pregnancy. Amniocentesis was done due to maternal age on the 17. week of pregnancy. Cultured amniocytes revealed mosaic karyotype 47,XYY(57%)/45,X(39%)/46,XY(4%). Ultrasound examination on 20. week showed normal fetal development with normal male genitals. Family decided to continue the pregnancy and apparently normal boy was born at term with birth weight 3864 g and length 52 cm. Detailed examination of placenta revealed karyotype 47,XYY in three different places of biopsy. Karyotypes from peripheral blood lymphocytes and skin fibroblasts showed the same mosaic karyotype as in amniotic fluid. Blood - 47,XYY(78%)/45,X(19%)/46,XY(3%) and skin - 47,XYY(76%)/45,X(22%)/46,XY(2%).
At the age of four months obstructive megaureter was diagnosed and operated. At the age of 7,5 months he a was normally developed boy. Ultrasound examination showed normal testis structure. The level of testosterone and hCG was normal. Laparoscopy and testis biopsy is planned at the age of one year.

P0327. II trimester serumscreening experience in Estonia.

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Maternal serum screening (double test) started in Estonia in autumn 1998 in Tartu, and only in 2002 in whole of Estonia. In 2003, altogether 70% pregnant women under 35 in Estonia were monitored.

The proportion of pregnancies screened varies, 11% in eastern Estonia and 90-95% in Tartu and Tallinn, the two biggest cities in Estonia. Laboratory testing of two serum markers (AFP, hCG) is performed in two public and one private laboratory.
During the 5 years (1998- 2003) altogether 25 500 screening tests were performed. Positive serum screening was indication for amniocentesis (fetal karyotyping) in 1076 (4,2%) cases, Chromosomal abnormalities were detected in 22 (1:49) cases, DS in 12 cases.
Screening for Down's Syndrome (DS). In this period (1998- 2003) 17,6 % of DS born women under 35 years have been diagnosed prenatally. In advanced maternal age risk group chromosome anomalies have been screened since 1995. During the 9-year period 61% of DS prenatally have been diagnosed. Incidence of Down Syndrome 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.
The decrease involves dominantly the advanced maternal age risk group. In order to reduce the rate even further, a greater percentage of pregnancies over a longer period of time in age group under 35 have to be monitored by maternal serum screening.
We thank laboratory LTKH and private laboratory HTI for allowing to use their data.

P0328. Double trisomy in spontaneous abortions

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More than 60% of the spontaneous abortions (SAs) in the first trimester have an abnormal karyotype. The most common abnormality observed is trisomy, but double trisomies are rare. Their frequency among karyotyped SAs varies in the literature from 0.21 to 2,8%.
We have found 5 cases of double trisomy among 343 SAs karyotyped in our laboratory (1.6%).

CASE	Gestation age (wks)	Maternal age	Paternal age	Gestations Gravida/ Para/ Abortions	Karyotype
1	10	45	36	G6/P2/A4	48,XX,+9,+21
2	7	37	39	G4/P2/A2	48,XX,+15,+22
3	8	40	49	G4/P2/A2	48,XX,+8,+21
4	9	36	35	G3/P2/A1	48,XY,+2,+8
5	11	40	42	G3/P1/A2	48,XX,+20,+22
Mean	9	39.6	40.2		

Acrocentric chromosomes were the most frequently implicated, as described in literature, but others like chr-16, are absent in our cases. The low gestational ages and the increased maternal ages observed are in accordance to the previously described.
In case 2, we determined the origin of trisomies 15 and 22 by comparing inheritance of microsatellite markers. The results were consistent with a maternal origin due to a meiosis-I error of both extra-chromosomes. This contrasts with the previously described origin of a double trisomy in SAs (Robinson et al,2001), which was consequence of both a maternal and paternal meiotic error. However it concurs with the idea that double trisomy, like single trisomy, is predominantly a result of meiotic errors related to increased maternal age. As the majority of the double trisomies were observed within couples with recurrent SAs, a higher risk for aneuploidy in some population can not be excluded.

P0329. Diagnosis of common aneuploidies in spontaneous miscarriages by Quantitative Fluorescent - PCR with STR markers.

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More than 50% of spontaneous miscarriages present chromosomal aberrations, being 96% of them numerical chromosome abnormalities. Conventional cytogenetic studies (karyotyping or FISH) encompass certain problems such as culture failure, infection or maternal contamination. For this reason we have incorporated the QF-PCR technique as it is a rapid, sensitive, accurate and reproducible diagnostic method.
We have carried out a rapid screening of numerical anomalies of chromosomes 21, 18, 13, X and Y in our clinical abortions. DNA was extracted from frozen tissues or cellular cultures. Two multiplex PCRs were performed with D21S1414, D21S1411, D18S535, D18S386, D13S631, D13S634, X22, HPRT and AMXY STR markers. In cases in which we did not detect those anomalies, they were screened with another set of chromosomal markers such as those for chromosomes 15, 16 and 22.
So far, 102 samples have been studied. Some of the cases, in which the cytogenetic diagnosis failed, were diagnosed of having different numerical chromosomal anomalies. In one specific case we could also confirm the clinical diagnosis of an hydatidiform mole. Those cases in which a cytogenetic result was obtained were used as controls to confirm our results, finding no discrepancies in any of them.
Thus, we consider QF-PCR as a complementary tool to cytogenetic studies in abortions, offering also the possibility of knowing the parental origin of the extra chromosomal material.

P0330. First trimester screening for trisomy 21 in more than 5000 pregnancies

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In prenatal diagnosis, the constant increase of maternal age over the last years has intensified the efforts to develop early non-invasive methods to screen for trisomy 21. In so called OSCAR clinics (One-Stop Clinics for early Assessment of fetal Risk), maternal age, fetal nuchal translucency, as well as maternal levels of free ß-human chorionic gonadotropin (f ß-hCG) and pregnancy-associated plasma protein-A (PAPP-A) are used as screening markers. We report about our own experience of combining these biochemical and ultrasonographic markers in more than 5000 completed pregnancies. The specific Down syndrome risk was calculated by using Fetal Medicine Foundation software. Karyotyping was offered to women with risks greater than 1:300. Until December 2003, a complete follow-up was available for 5000 pregnant women. 107 chromosomal defects (2,1%) including 48 cases of trisomy 21 (0,96%) were detected. 42 out of 48 Down syndrome pregnancies were found resulting in a detection rate (DR) of 87,5% and a screened positive rate (SPR) of 12,5%. The DR and the SPR both increased with maternal age; they were 75% and 8%, respectively, for women aged 34 years and younger, and 94% and 23%, respectively, for women aged 35 years and older. After the introduction of the first trimester screening to our clinic, the numbers of invasive genetic testings decreased especially in women between 33 and 35 years of age. In our experience, the combined first trimester screening has a high sensitivity, an acceptable specificity and the advantage of a risk assessment early in pregnancy.

P0331. Validation of QF-PCR assay for detection of aneuploidy in an Australian population.

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A sixteen marker, two-reaction assay was developed using a combination of previously reported markers along with additional X/Y chromosome markers to improve the determination of sex chromosome aneuploidies.
Prospective analysis was carried out on 544 samples comprising CVS, amniotic fluids and products of conception. The results of the quantitative fluorescence PCR were confirmed by cytogenetic karyotype analysis.
Of the samples analysed, 514 (94.5%) showed no allele peak imbalances for the markers representing chromosomes covered by this assay. Fourteen cases of trisomy 21, six cases of trisomy 18 and three cases of trisomy thirteen were correctly identified. Seven cases involving sex chromosomal aneuploidies were also detected.

Results were achieved within 24-48 hours for the majority of samples, showing that QF-PCR is a suitable alternative to FISH for rapid aneuploidy screening. This study allowed us to investigate the heterozygosity of the chosen markers in our ethnically diverse Australian population, which is useful in predicting the amount of further testing which will be required due to lack of sufficient chromosomal information.
Our results also indicate that the QF-PCR assay is more versatile than FISH for problematic samples as well as being more cost effective for general use.
Test referral patterns in the initial introduction phase of this test and further ideas on future refinements of the test/service in order to improve sensitivity/informativity shall be discussed.

P0332. Evaluation of clinical application of a real-time PCR method with multiplexed "mini-fingerprinting" to preimplantation genetic diagnosis (PGD) in 34 cycles at risk for sickle cell and thalassaemia syndromes

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Beta-thalassemia and sickle-cell syndromes are amongst the commonest monogenic disorders worldwide, and PGD represents an alternative to prenatal diagnosis (PND) for them. PCR-based PGD methods have inherent difficulties, but real-time PCR with hybridization probes (Lightcycler™), represents a potentially sensitive, accurate and rapid means for genotyping single-cells. For application to PGD we established a real-time PCR protocol capable of detecting the majority of common β-globin gene mutations worldwide.
To monitor chance contamination we multiplexed this to allow parallel analysis (in an automatic sequencer) of two polymorphic microsatellite markers, GABRB3 and D13S314. Clinical application in 34 PGD cycles (25 couples, 17 different β-globin mutation interactions) gave genotype results (available within 5-6hours) at all 3 loci in 248/290 single blastomeres (86%). ADO at the β-gene locus was detected in 14% of blastomeres, although transfer of affected embryos is precluded by transferring only those with normal allele(s) detected (141/248 (57%) definitely unaffected). Contamination was detected (non-parental microsatellite alleles) in 3 cycles (6 blastomeres), including 3 embryos with unaffected β-genotype (not transferred). Transfer of at least one unaffected embryo/cycle (total 112/34cycles), initiated 12 pregnancies. Four were lost, but PND in 7 confirmed unaffected status in 4 twin pregnancies and 3 singleton pregnancies. Five pregnancies have gone to term (8 healthy babies), including one without PND (parental choice). Additionally, PGD β-genotypes were confirmed on re-analysis of 50 non-transferred embryos. This multiplex real-time PCR protocol appears to fullfill all requirements for a clinical PGD method ie sensitivity, accuracy, speed and relative simplicity, with internal monitoring for contamination.

P0333. Prenatal sonographic findings in 5 cases of trisomy 22

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We describe 5 cases of trisomy 22 detected prenatally on second-trimester sonography. Fetal abnormalities shown on sonography includes nuchal thickening, generalized skin edema, an atrioventricular septal defect, tetralogy of Fallot, ascites, a single umbilical artery, hydronephrosis, microcephalus and Dandy-Walker malformation. All 5 fetuses had karyotypes indicating trisomy 22.
Our literature search revealed only 2 reports of second-trimester sonographic diagnosis of trisomy 22. We will compare our cases with the published cases.

P0334. Prenatal diagnosis in Myotonic Dystrphy type 1 (DM1): thirteen years of experience

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Myotonic dystrophy type 1 (DM1) is the most common form of adult muscular dystrophy. It's an autosomal dominant disorder characterised by myotonia, progressive muscle weakness and wasting, cataracts, frontal balding and cardiac conduction defects. The severity of the disease is highly variable and patients can be divided into four groups: asymptomatic, mild, classical and congenital forms.
Myotonic dystrophy is characterised by anticipation (earlier age of onset and more severe clinical course in successive generations in a DM1 family).
The molecular basis is the expansion of an unstable CTG repeat located into the DMPK gene on chromosome 19.
Genetic counselling for myotonic dystrophy is difficult and complex, owing to the extreme variability of the disorder, in both severity and age of onset, the anticipation and the influence of the sex of the affected parent.
We have carried out 130 PND, most of which (70%), the mother is the transmitting progenitor, with expansions ranging from 65 -1166 CTG. The remaining 30% of PNDs are of paternal origin, ranging between 42-600 CTG. 48% of PND where positive for the DM1 mutation, with expansions between 45 - 3666 CTGs in foetuses.
In maternal transmissions we detected a predominance of expansions, with few contractions or no-variations. All the congenital cases are of maternal origin, and according to our data, if a female has one congenital infant, all her next affected descendants will be congenital cases.
The contractions or small variations are predominant in paternal transmissions, although, the foetuses inherited alleles clearly into the pathological range.

P0335. Amnio-PCR in routine use

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Amnio-PCR has become routine adjunct to conventional prenatal cytogenetic analysis at our institution. 53 per cent of all prenatal procedures (1151/2137) have undergone this procedure since its introduction in January 2003. Amnio-PCR was offered at 1/ antenatal screening positivity, 2/ fetal morphological defect, 3/ gestational age over 20th week, 4/ discoloured amniotic fluid , 5/ mother´s anxiety but not at pure age indications. If defined Down syndrome risk was the main reason for prenatal diagnosis only chromosome 21 together with XY specific markers was used. 54 per cent of Amnio-PCRs (623/1151) belonged to this group. Common (X, Y, 21, 18 and 13) aneuploidies were searched for at the rest (528/1151) using different marker combinations according to informativity. Conclusion: Chromosome 21 specific Amnio-PCR can quickly reveal stress of false screening positive mothers. Together with finding of a serious fetal morphological defect the positive Amnio-PCR can be indication for termination of pregnancy. Amnio-PCR can replace short term CVS cultivation.

P0336. Cvs Study Of Spontaneous Abortions In The First Trimester.

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Chromosomal abnormalities are the most important cause of spontaneous abortions (SAs). However, the cytogenetic study is not always possible, thus making difficult the subsequent genetic counselling.
The collection of villus samples before surgical evacuation has been described as a good method to obtain a karyotype.
Transcervical villus samples were obtained from 61 SAs. Forty-eight were valid for this study according to the following criteria that ensure reliability: 1) pregnancies up to 90 days, 2) obtention of results from the direct and long term culture study, 3) concordance between

both studies. Seven of the 48 pregnant women underwent assisted reproduction techniques (ART).
An abnormal karyotype was found in 52% of the 48 cases.
The anomaly most frequently found (48%) was the trisomy (specially tri16), followed by Turner Syndrome (20%), unbalanced translocations (16%), triploidy (12%) and presence of a marker chromosome (4%). The mean maternal age in those cases in which a normal karyotype and a trisomy were observed were 34.8 and 37.1 years old respectively.
The anomalies found are in accordance with the low gestational age and the advanced maternal age.
Analyzing separately the 7 cases coming from ART, the proportion of cases with an abnormal karyotype was 57%. The anomalies observed were Turner Syndrome (50%), tris16 (25%) and tris17 (25%). The mean maternal age in those cases presenting a trisomy was 38.
Although there are a few cases from ART there is no significant difference in the incidence of anomalies and the mean maternal age.

P0337. Prenatal diagnosis in the population of Emirates (UAE) - A report of 459 cases.

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Chromosomal anomalies usually invoked as major cause of birth defects, repeated fetal loss and bad obstetric history, or when they have an increased risk of carrying a fetus with chromosomal abnormalities as assessed from various etiological parameters including advanced maternal age. We present here our experience in investigating 459 cases referred for prenatal diagnosis, by culturing amniotic fluid samples between 16-20 weeks of pregnancy, in the heterogeneous population of United Arab Emirates.
Chromosomal abnormality was confirmed in 17(3.7%) of the cases. Trisomy 21=6 (one mosaic), Trisomy–18=2, 1 case each of XXY, XYY, mosaic 46,XX/45,X. and one case of tetraploidy 92,XXXY. Isochromsome X was found in case. Robertsonian translocation (centric fusion) was found in 4 cases. The cytogenetic abnormalities in this preliminary study emphasizes the importance of antenatal diagnosis in high risk pregnancies and in the prevention of birth of children with chromosomal abnormalities.
Prenatal diagnostic services is not available in most of the Arab countries for socio-cultural reasons. There is a great need to establish a Registry of high risk families, particularly with balanced translocation (heterozygous) parents and sibs, to provide proper genetic counselling and prevent birth of children with major chromosomal abnormalities, often lethal.

P0338. Difference in nuclear DNA fragmentation in sperms between IVF and ICSI patient

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Male factor infertility patients can have abnormalities of their sperm nuclei and or display high levels of loosely packaged chromatin and damaged DNA. Standard sperm characteristics are used to choose the procedure, IVF or ICSI, but are poor predictors of the success. On the contrary, sperm genome quality has been emphasized for several years as playing a major role in early embryogenesis, thus in the success of IVF or ICSI attempt. The aim of this study was to analyse the correlation between the percentage of spermatozoa with fragmented DNA and fertilization rates after IVF and in ICSI.
A total of 60 semen samples was collected. 30 men underwent semen analysis for IVF andrology assessment and 30 men for ICSI. Semen samples (50000 sperms per sample) were diluted and resuspended in PBS. The sperms were stained with propidium-iodide as previously reported (Nicoletti et al., 1991), with slight modifications. Flow cytometry analysis was performed using the fluorescence activated cell sorter equipped with an argon laser. Spermatozoa of poor quality, as used for ICSI, contained a significantly higher amount of fragmented DNA than spermatozoa from men in IVF program (Median: 25.3% vs. 8.8%; Wilcoxon test: p=0.003). There was a positive correlation between the percentage of fragmented DNA and semen samples with poor quality.
We conclude that the DNA fragmentation is like other standard sperm parameters a poor predictor for the rate of fertilization as long as we can generate a selective advantage by the ICSI treatment.

P0339. Prenatal diagnosis of a de novo terminal deletion of chromosome 11q

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A 34-year-old woman presented for amniocentesis at 17 weeks of gestation due to oligohydramnios and reduced movements of the fetus. Cytogenetic analysis of cultured amniocytes and parental lymphocytes revealed a de novo terminal deletion of chromosome11q23-ter in the fetus (Jacobsen syndrome, OMIM 147791). Repeated ultrasonography at 20 weeks showed moderate cerebral ventricular dilatation and a two-weeks growth retardation. Clinical features of Jacobsen syndrome were discussed with the couple during genetic counselling. The couple decided to terminate the pregnancy at 20 weeks of gestation. On fetopathological examination of the female fetus, no externally visible dysmorphological features were recorded. Measured dimensions (145 mm sitting height, 27 mm maximal foot lenght) corresponded to fetuses at 18/19 weeks of gestation. Examination of the internal organs did not disclose prominent malformations but only a few minor anomalies (Meckels‘ diverticulum, adhesion between the gall bladder and the transverse colon, bilaterally bilobed lungs without further signs of situs anomalies). The brain appeared normal on gross morphological examination. Due to poor preservation of the brain, measurements of the ventricles could not be performed. The molecular breakpoint on chromosome 11q was further mapped by a quantitative PCR approach with SYBRgreen detection. A genetic marker analysis was performed to show the parental origin of the deleted chromosome. Patients with Jacobsen syndrome show a wide spectrum of phenotypes depending on the breakpoint. The phenotype/breakpoint correlation in the present study is discussed with respect to cases reported previously in the literature.

P0340. Differentiation of foetal genotype and maternal contamination of amniotic fluid by innovated QF PCR.

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QF PCR is routine prenatal diagnostic technique to rapid, cheep and reliable sex and common aneuploidy determination. The method of analysis DNA from amniotic fluid permits unambiguous diagnostic conclusion.
However the interpretation in cases of mosaics or maternal contaminations is more complicated and it is difficult to assess ratio of particular genotypes exactly. Especially if the genotypes differ in several orders. In those events the mosaic can be missed or undervalued.
To eliminate and decrease of non exact assessment mosaics or maternal contamination the innovated (I)QF PCR was introduced in our department. This method makes corrections of QF PCR by manual real-time PCR. IQF PCR was evaluated and calibrated in D21S1411 and Penta D loci using 0,2% - 100% prepared mosaics. The aim of our study was to assess and optimise IQF PCR quantitative possibilities to invasive and non-invasive prenatal diagnostic purposes. Results proved that the sensitivity of DNA quantity change detection is a few percent and confirmed assumption that the same samples can demonstrate different fluorescent ratio values in different PCR cycles which leads to misrepresentation of results. IQF PCR refines molecular diagnostics based on analysis of PCR products. Its sensitivity can be important in foetal DNA and DNA chimerism analysis.Supporting grant: *MSMT CR CEZ J1498:15110000006*

P0341. Possibilities of fetal DNA analysis on capillary electrophoresis in non-invasive prenatal diagnosis.

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Pre-implantation diagnosis and non-invasive prenatal diagnosis of foetal nucleic acid in maternal tissues are in the fore front of taking prenatal diagnosis to early stage of pregnancy.
Then foetal nucleated cells or free extra-cellular DNA or bound RNA in maternal plasma are used. The concentration of free foetal DNA is approximately thousand times higher than DNA amount in intact foetal cells circulating in maternal peripheral blood.
For diagnostic purposes precise diagnosis and quantification of foetal DNA is crucial.
Determination of foetal sex from maternal serum by real-time PCR of Y specific sequences in male foetuses is relatively well developed and reliable, but detection of foetal aneuploidies from maternal serum is complicated. The main objective of this study was to assess and optimise quantitative possibilities of innovated QF PCR in detection of free foetal DNA in maternal serum. Artificial DNA mosaics, DNA from plasma, amniotic fluid, and maternal and paternal peripheral blood were analysed by innovated QF PCR in D21S1411, D21S1446, AMELY and TSPY loci.
Variance assessment of mean RFU values of alleles ratio proved increase or decrease of those values compare to normal lineage.
In one case free foetal DNA was proven in TSPY and twice in D21S1446 loci.
Innovated QF PCR enables mosaics differentiation on level of several percentages. To decide if foetal DNA diagnostics is feasible for distinguishing disomy/trisomy by quantification STRs from desired chromosome the curve of normal foetal DNA concentrations during normal pregnancies must be compiled.
Supporting grant: *MSMT CR CEZ J1498:15110000006*

P0342. Evaluation of molecular methods applicable to PGD for monogenic disorders

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Pre-implantation genetic diagnosis (PGD) for monogenic diseases such as Cystic Fibrosis (CF) and Duchenne Muscular Dystrophy (DMD) involves PCR amplification from a single cell from a pre-implantation embryo. As single cell PCR is prone to allele drop-out (ADO), amplification failure of single loci (AF), and contamination, tests must be set up such that they minimise the occurrence of these events, as well as essentially eliminate the risk of misdiagnosis if they do occur. Additionally, as embryos have a limited viability in culture, the diagnosis must be obtained within as short a time as possible.
In establishing PGD for monogenic disorders, an evaluation of current molecular methods available for mutation detection in the context of a PGD lab was made. The methods chosen for mutation detection were required to address the issues of ADO, AF, contamination and time constraints, as well as be economically feasible. The evaluated methods included allele-specific PCR, single primer extension, melting curve analysis, the ligase detection reaction, restriction digestion, and the use of microsatellite markers. Certain PCR parameters were also investigated.
The most important strategy proved to be the inclusion of informative microsatellites that are co-amplified alongside mutation specific loci in a single cell multiplexed reaction. These microsatellites not only confirm the diagnosis, they also provide an internal control for PCR and maternal contamination. The evaluation of molecular methods for the detection of CF, DMD and Beta Thalassemia mutations will be presented, with particular reference to the unique requirements of single cell analysis for PGD

P0343. Prenatal molecular diagnosis of skeletal dysplasia syndromes

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We present 41 cases of skeletal dysplasias diagnosed prenatally by ultrasound and characterized by gene mutation detection. Detailed clinical and molecular data as well as the results of pathological examinations are available for each case and will be presented.
We could diagnose 13 cases of thanatophoric dysplasia type I, 3 cases of thanatophoric dysplasia type II, 2 cases of achondroplasia, 2 cases of Apert syndrome, 1 case of Pfeiffer syndrome, 7 cases of osteogenesis imperfecta, 2 cases of hypochondrogenesis, 1 case of achondrogenesis, 2 cases of diastrophic dysplasia, 2 cases of hypophosphatasia, 2 cases of campomelic dysplasia, 1 case of Ellis-van-Creveld syndrome and 1 case of oto-palato-digital syndrome type II. In this last case X inactivation studies in the mother confirmed the diagnosis and an indirect molecular genetic diagnosis could be offered in the next pregnancy revealling a non affected boy. In12 other cases an early direct prenatal molecular genetic diagnosis could be performed in the next pregnancy.
The identification of the underlying mutation in skeletal dysplasia syndromes is essential for genetic counselling for the ongoing and further pregnancies and in order to offer an early prenatal diagnosis in the next pregnancy.

P0344. Prenatal diagnosis of mosaicism for trisomy 21 and double trisomy Y and 13

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We report the case of a 30-year-old primigravida who underwent the amniocentesis at 17 weeks gestation because of increased nuchal translucency (3,8mm) detected in the 12 week of pregnancy. The cytogenetic examination revealed an equal mosaic of cells with karyotypes 47,XY,+21 and 48,XYY,+13 in two independent culture flasks. The pregnancy was terminated at the 20 week; there was no evidence of relevant external either internal developmental defects. Post-mortem tissue cultures of the foetal skin and the placenta were established. Foetal karyotype was found 47,XY,+21 with a minority (4%) of 48,XYY,+13 cells meanwhile in placenta the ratio 47,XY,+21 / 48,XYY,+13 was 1:2. The origin of supernumerary chromosomes was determined using QFPCR to be paternal, arisen from the second meiotic division or (more probably) due two postzygotic errors. This is the first report of this kind of mosaicism.

P0345. Prenatal diagnosis in a twin pregnancy of spinal muscular atrophy with respiratory distress (SMARD)

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Autosomal recessive spinal muscular atrophy with respiratory distress type1(SMARD1;OMIM604320) is the second anterior horn cell disease in infants in which the genetic defect has been defined. SMARD1 results from mutations in the gene encoding the immunoglobulin μ-binding protein 2 (*IGHMBP2*), located on chromosome 11q13.
We performed molecular prenatal diagnosis of SMARD in a twin gestation at 11 weeks on chorionic villus samples (CVS). Parents were both heterozygote for a 1540G→A transition in exon 11, causing a glutamic acid to lysine substitution(E514K). Molecular analysis was performed on CVS DNA by automated sequence analysis, evidencing a foetus heterozygotes for E514K mutation and one homozygotes wild-type. The intrafamilial segregation of the mutation was also demonstrated by primer-induced restriction analysis. Haplotype analysis in both CVS samples confirmed the results obtained with mutation screening end excluded any maternal contamination.
Kariotype analysis was performed on cultured CVS evidencing dichorionic twins of different sex: in one of these, an extra chromosome marker mosaicism was evidenced. For this reason amniocentesis was performed at 17 week of gestation and a normal kariotype in both foetuses was obtained, demonstrating the presence of a confined placental mosaicism.
Also molecular analysis, performed on cultured amniocytes, confirmed the genotypes for *IGHMBP2* locus.At birth molecular tests on peripheral blood samples gave the same results as above.
This is the first case of molecular prenatal diagnosis of SMARD1

reported up to date. SMARD1 diagnosis results of particular importance for offering accurate genetic counselling and for initiation of mechanical ventilation of an affected infant.

P0346. Running CGH on very low DNA quantities

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Pre implantation genome diagnosis (PGD) demands a method which meaningfully analyses the presence or absence of as many human chromosome types or parts as possible. The use of specific FISH probes for PGD reaches limits which are caused by a low number of available colours. Different working groups have already used a CGH with blastomeres or with polar bodies successfully. Both causes, that the CGH provides a meaningful result even if it starts out from very few DNA. Thus, the small quantity of DNA must be amplified and marked at first. Here we would like to introduce the effectiveness of the CGH outgoing from a small DNA quantity, amplified by degenerated oligo-primed (DOP)-PCR before. In order to this, we compare two known genomes in a CGH by an increased demand of sample DNA between one to four nuclei each.
We have examined two postnatal striking karyotypes with one derivative chromosome each by microdissection. The first, female karyotype, showed a partial trisomy 16q21-qter; 46,XX, der add (14)(q). rev ish der t(14;16)(qter;q21). The second, male karyotype, an abbreviated Y chromosome pointed; 46,X, der(Y). rev ish del(Y)(q11.2-qter). The comparison of both genomes let expect an exactly defined result. We have isolated nuclei of both cell suspensions by microdissection, and amplified them separately with DOP-PCR. The amplification products of both cases were marked and hybridised for a CGH with each other.

P0347. Prenatal first trimester screening in Saint-Petersburg.

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Since February of 2003 all pregnant women, irrespective of age, can have a new Down syndrome screening test in Saint-Petersburg. 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 nasal bone. The effectiveness of combined ultrasound and biochemical screening in the first trimester of pregnancy will be compared with second trimester screening results. It is important to make such analysis due to lack of such investigations in russian literature. We investigated 637 women at 10-13 weeks of pregnancy. We use ultrasound scanner Aloka SSD-2000 and system of prenatal screening Life Cycle (Pribori Oy, Moscow). In 26 of 637 patients have NT 2,5 mm and more, 10 of them have chromosomal diseases. In 4 of 637 patients there was absence of fetal nasal bone, 3 have chromosomal diseases and one fetus has severe malformations. In 64 of 637 have increased risk of fetal chromosomal pathology due to results of biochemical screening, 7 of them have chromosomal pathology. Totally we find out 4 fetuses with Down syndrome, 2 fetuses with Edward's syndrome, 2 fetuses - 47,XXY(X), 4 fetuses with Turner syndrome, one fetus with triploidy. Combined screening in the first trimester of pregnancy increases the effectiveness of detection of fetal chromosomal diseases.

P0348. Experience of prenatal diagnosis for sickle cell disease amongst Africans in Geneva

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Direct molecular testing for sickle cell disease (SCD) was introduced in Geneva in 1983. We retrospectively evaluated the clinical and laboratory records of all referrals for prenatal diagnosis of SCD between 1983 and 2003.
A total of 29 couples and 3 singles were seen for genetic counselling of SCD. The majority was of West/Central African origin (97%). Six couples came more than once, resulting in a total of 51 consultations. At first referral, 24 of 29 couples (83%) had an ongoing pregnancy, 37.5% in the first trimester. Nine couples already had an affected child; 11 further at-risk couples were identified by genotyping. All but one at-risk couples (19/20, 95%) accepted PND in principle; however, only 14 of these (74%) requested foetal testing at the first opportunity.The remaining 5 couples chose to have at least one pregnancy without PND, resulting in the birth of 2 affected children. Explanations included fear of abortion (2/5), religious, ethical or personal reasons. In all, 30 PND (18 CVS, 12 amniocenteses) were performed. Seven affected foetuses were identified (23%); 4 medical abortions were requested.
In conclusion, we believe 1) that potentially at-risk couples in this population are under-referred; 2) that they are commonly referred too late, either during pregnancy or after the birth of an affected child; 3) that the decisions to request prenatal diagnosis or to terminate affected pregnancies are very complex. This study reveals the need for further investigation of attitudes concerning SCD and PND in African communities.

P0349. Hb Neapolis (Dhonburi) causing silent beta-thalassemia in a family of Northern Iran

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Thalassemias are the most common heredity disease in Iran, resulting from synthesis defects in one or more hemoglobin subunits. The majority of patients suffers from beta-thalassemia, but cases with microcytic hypochromic anemia and normal electrophoresis pattern are suspected to be alpha- or silent beta-thalassemia.
A family from the northern part of Iran, which is an area highly prevalent for thalassemias, was referred to us for prenatal diagnosis. The hematological data of the father indicated a pattern of beta-thalassemia minor. Reverse-hybridization analysis for the most common beta-globin mutations identified IVSII-I G>A in the heterozygous state. The maternal laboratory data (MCV: 77.8 fL, HbA2: 3.0%, HbA: 96.5%) indicated a case much more compatible with alpha-thalassemia. Iron-deficient anemia was ruled out, and common alpha-thalassemia point mutations and deletions were investigated. As no mutation was detected, chain synthesis was performed showing an alpha/beta ratio of 2.1, which was in the range of beta-thalassemia minor. DNA sequencing of the entire beta-globin gene identified a heterozygous GTG>GGG (Val>Gly) mutation in codon 126, also known as Hb Neapolis (Dhonburi). Prenatal diagnosis of the fetal DNA showed an absence of both IVSII-I and the cd 126 mutation.This result demonstrates the importance of screening mild microcytic hypochromic individuals for both alpha- as well as silent beta-thalassemia mutations.

P0350. Zygosity studies of twins conceived by in vitro fertilization

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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 50 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. Fingerprinting analysis was made and 9 genetic markers covering 6 chromosomes were analyzed. The results of analysis show that in two cases blood chimerism may be considered, which suggests that the influence of in vitro fertilization on early embryonic development requires further investigation.

P0351. Complex chromosome rearrangements in prenatal diagnosis

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Complex chromosome rearrangements (CCR) are defined as reciprocal exchanges between three or more chromosomes. It has been observed that most CCR carriers are female. CCr are very rare, with the risk for phenotypic abnormalities increasing as the number of chromosomes and chromosomal breaks involved in the rearrangement increases. The normal phenotypes of such balanced carriers CCRs suggests that presented breakpoints are not in gene regulatory or critical functional chromosomal regions.
We report pregnancy outcomes and prenatal diagnosis of three cases with CCRs.
A de novo complex chromosomal rearrangement involving chromosomes 4,7 and 14 was detected prenatally at 18 weeks of gestation made for advanced maternal age. Using routine cytogenetic analysis of cultured amniotic fluid cells with GTG-banding, it was found interstitial deletion of band 4p15.2→15.32 and reciprocal translocation t(7;14)(p13;q11.2), but it was impossible to be correct karyotypically determined. Fluorescent in situ hybridisation (FISH) with centromeric and „painting“ chromosome - specific probes was used to explain this rare chromosome rearrangement. The chromosome 4 paint reveald chromosome 4 material inserted in derived chromosome t(7/14).
In two cases mothers had a balanced complex chromosome rerrangement, detected through abnormal offspring and spontaneous abortions. The karyotype of CCR carrier was determinated as 46,XX, t(1;8;9)(q25q41;q13;p22), and the other as 46,XX,t(4;8;15)(p15p16;p 11;q13). Although balanced carriers of CCR have a relativly high risk of producing unbalanced gametes, prenatal diagnosis in those cases showed that thay can have healthy offspring.

P0352. De novo chromosome rearrangements in prenatal diagnosis

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Balanced or unbalanced structural abnormalities may be inherited from a carrier parent or may occur as de novo rearrangements. When the abnormality occurs as a de novo event, the risk for genetic disease or phenotypic effects is increased, even when the rearrangement appears balanced.
The aim of this study was to examine the incidence and clinical outcome of de novo chromosomal aberrations.
Prenatal cytogenetic analyses were performed in 20,506 pregnancies at our hospital from 1979 to 2004. Karyotyping by G-banded metaphase chromosome analysis of cultured amniotic fluid samples was performed using standard techniques.
In our study we found 31 case of the de novo chromosomal rearrangements in 20,506 cases of prenatal cytogenetic analysis. Out of the 31 de novo aberrations, 14 had balanced translocations; 13 had balanced reciprocal translocations and 1 had balanced Robertsonian translocation. Of 17 unbalanced de novo aberrations, 10 had one structurally abnormal extra chromosome, 1 had unbalanced reciprocal translocation, 5 had unbalanced Robertsonian translocation, and 1 had a de novo inversion. All unbalanced translocations were terminated.
The outcome of pregnancies and the clinical follow up of rare cases with apparently balanced de novo translocations are very helpful for a better risk assessment in prenatal genetic counselling.

P0353. An Analphoid Marker Chromosome Inv Dup(15)(q26.1), Detected During Prenatal Diagnosis and Characterized via Chromosome Microdissection.

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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 labour at 29 weeks gestation, a dysmorphic infant was delivered, with flat nasal bridge, short palpebral fissures, micrognathia, high forehead, low-set ears, telecanthus and corneal dystrophy. Additional folds of skin were present behind the neck, and feet and fingers and toes were abnormally long. The child died at age five days, after two days in renal failure. The origin of the marker chromosome was subsequently identified from a cord blood sample, 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 were positive for the telomeric TTAGGG probe. These data, plus the G-banding pattern, identified this as an analphoid, inverted duplicated marker, lacking any conventional centromere. We discuss the aetiology and clinical effects of this marker, comparing it to the few other reported cases of “tetrasomy 15q” syndrome.

P0354. Oocyte aneuploidy testing for 8 chromosomes by first and second polar body FISH analysis.

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Introduction: Polar body (PB) analysis during ART cycles identify oocytes with chromosomal non-disjunctions. By fluorescence in situ hybridization (FISH) it is possible to test first and second PBs in a first round with 5 chromosomes (13, 16, 18, 21 and 22) followed by a second round with 3 chromosomes (8, 15 and X). The results are completed prior to pronuclear fusion. With the standard 5 chromosome analysis, we detect approx. 35% of trisomies. By investigating 3 more chromosomes the detection rate is increased up to 60% of all trisomies found in miscarriages.
Material and methods: Out of a total of 48 PB-cycles we were able to extract 464 polar bodies (353 first and 121 second PBs) from 367 oocytes by standard methods. After pre-treatment they were hybridized with the 5 probe set. 60 PBs were rehybridized in a second round with the additional 3 probe set .
Results: Altogether 59% of the evaluated oocytes (161/272) were aneuploid. Aneuploidy for the additional tested 3 chromosomes were identified for 10 of the 28 oocytes (36%).
Conclusion: The extension of PB analysis for 3 more chromosomes is achievable even in the very tight time frame of the German Embryo Protection Law. According to our preliminary results it is worth while investigating 8 chromosomes for aneuploidy testing of oocytes, even if that means a tougher schedule. Extended data is necessary to prove that the benefit of excluding non-disjunction for 8 chromosomes results in an increased baby take home rate.

P0355. Oppenheim‘s dystonia: The challenging genetic counseling associated with prenatal diagnosis

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Oppenheim’s dystonia (OD) is an autosomal dominant (AD) movement disorder with a variable phenotype and penetrance of 30-40%. It is characterized by early onset, sustained, involuntary muscle contractions that cause significant morbidity. The identification of the causative GAG deletion in the DYT1 gene enabled prenatal

diagnosis (PND). We have ascertained 53 OD affected individual within 28 families, confirmed molecularly. Five couples requested PND in which three men were non-manifesting carriers and two women were affected with OD. Comprehensive genetic counseling was provided twice by a medical geneticist, genetic counselor and a movement disorder specialist before prenatal testing and when results were delivered. Seven PNDs were preformed. Three fetuses of three coupes were found to carry the GAG deletion. Genetic counseling in OD is challenging and presents intellectual, emotional, and moral dilemmas. The fact that only one parent is "responsible", that inheriting the mutation does not necessarily mean being affected, and the modern treatments that allow most patients a relatively good quality of life, contribute to its complexity. A multidisciplinary approach and possibly preimplantation diagnosis are suggested to best meet the needs of couples at risk.

P0356. Lethal forms of bone dysplasias - prenatal diagnosis, obstetrical management

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The main objective of this study is to present the importance of routine fetal ultrasound scan in the prenatal diagnosis of three types of lethal bone dysplasias.
Material and method: Echographic examination was done according to the protocol for fetal morphology. For all the cases the examination was followed by termination of pregnancy, radiologic and pathologic examination of the fetus. The final diagnosis was established by the geneticist.
Results: Team examination of the cases allowed the diagnosis of the following lethal bone dysplasias: 1) Achondrogenesis type I diagnosed at 24 weeks gestation, first child of a 21 years old woman; 2) Jarcho-Levin spondylo- thoracic dysplasia diagnosed at 32 weeks gestation, third child of a 29 years old woman; 3) Thanatoforic dysplasia type I diagnosed at 26 weeks gestation, second child of a 27 years old woman. Detailed presentation of the cases will be included.
Conclusions: In the cases mentioned above routine echographic examination done for fetal morphology identified major bone abnormalities that indicated the profilactic termination of pregnancy. Multidisciplinar examination of the fetuses allowed the identification of 3 rare variants of lethal bone dysplasias.

P0357. 14 years experience in prenatal diagnosis. Report of 3500 PND tests

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With advances in cytogenetics, molecular genetics and Prominent improvement in ultrasonography, PND tests became a popular and acceptable mean for prevention of genetic and congenital disorders at early 1970s. We performed the first amniocentesis for chromosomal aberration in summer of 1986, then there have been steadily increasing numbers up to 350 tests per year at present, totalling 2500.
The higher risk 12% belong to the couple in whom one is carrier of a balanced translocation, followed by 5.8% risk in mothers with advanced maternal age and history of offspring with chromosomal abnormality. The 2nd attempt was PND for hemoglobinopathies started at the end of 1990, since then 869 PNDs tests have been performed for β, α thalassemia, sickle cells and other hemoglobinopathies, using ARMS, RFLP and DNA sequencing when necessary.
PND for Fragile X Syndrome was established next, using cytogenetics and DNA analysis; testing more than hundred families, and 13 PND tests. PND for metabolic disorders with collaboration of Clinical Genetics. Dept of Erasmus University was established in 1999, 82 PND tests have been performed for 45 Mucopolyschardoses, 20 Lipid storage and 19 micromolecular. PND tests for neuromuscular diseases including 39 DMDs and 50 SMAs by molecular analysis have been performed so far. Eight PND test for skin lesions (Xeroderma Pigmentosa) and one PND test for Ataxia Telengectasia have been done, the results and details of findings are being presented.

P0358. Prenatal diagnosis of a 5p trisomy resulting from familial balanced reciprocal translocation including a de novo extra structurally abnormal chromosomes (ESACs)

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We report a case of partial trisomy 5p combined with marker chromosome. To the best of our knowledge, this is the first reported case of a 5p trisomy involved the region of 5pter5p12, resulting from the rearranged chromosomes in a familial balanced reciprocal translocation. Cytogenetic analysis of amniotic fluid cells at 17 weeks gestation showed 47,XX, der(13)t(5;13)(p12;p11.2),+mar. The proband's mother was a carrier of a balanced reciprocal translocation with 46,XX,t(5;13)(p12;p11.2). NOR banding, CBG banding, RBG banding, and high resolution banding were performed to precise characterization. Fluorescence in situ hybridization (FISH) analysis showed that the marker chromosome was a dicentric chromosome derived from chromosome 15. No physical abnormalities were shown in the targeted fetal ultrasonography examination. The FISH analysis was useful to elucidate the nature of these rearrangements.

P0359. Prenatal diagnosis of a partial trisomy 11q21-qter

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We report a new prenatal diagnosis case of chromosome 11 partial trisomy in a foetus from a first pregnancy of healthy parents. Amniocentesis was performed after 23 week's gestation because of ultrasonographic detection of a cystic hygroma with ascites and oligoamnios.
Foetal karyotype was performed after amniotic liquid culture according to conventional techniques. An additional chromosomal materiel was detected in one chromosome 13.
Parental karyotype showed a balanced reciprocal translocation t(11;13)(q21;qter) in mother chromosomes. So the foetus had the derivative chromosome 13 of this translocation and he has consequently a partial trisomy 11q21-qter.
In spite of genetic counselling, the couple decided to carry on the pregnancy. The infant was born at a correct term, by a caesarean section for an acute foetal sufferance. At birth the infant presented an immediate and sever breath distress with an Apgar score of 3/6. He had dead at the 11th hour of life.
Foetopatholgic exam, revealed hypotrophic and dysmorphic mal sex foetus. Extremities examination showed an arthrogriposis with limbs amyotrophy.
Brain examination found a polymicrogyria and no visceral anomalies were detected.Infant with trisomy11q present habitually less severe phenotypes and, arthrogriposis was not described to be associated with this chromosomal abnormality.

P0360. The Report of the National Prenatal Diagnostic Reference Laboratory in Iran for Thalassemia Syndromes

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Thalassemia is a major health problem in Iran with an estimated two millions carriers. Genetic counseling and prenatal diagnosis is the only way for prevention of this disease. Iranian scientists and Ministry of Health have developed a national thalassemia prevention program, aimed at controlling thalassemia in Iran. According to the National Thalassemia Prevention Program, which is consist of many different peripheral laboratories and two reference laboratories, our aim is to improve our abilities in diagnostics. Here the activities of the national reference laboratory (GRC) during the last five year's are presented. Sixty five families at risk of thalassemia were tested with direct and indirect mutation detection methods including ARMS-PCR,

reverse hybridization assay and multiplex PCR. DNA sequencing was performed for negative cases. Prenatal diagnoses were performed for 34 cases. Of these 5 fetuses were found to be normal, 10 homozygous or compound heterozygous for both alleles and 19 heterozygous cases were identified. Also a total of 114 DNA samples with unknown β-globin gene mutations. Referred to our laboratories were analyzed. Mutations in 93 cases were identified. Including 14 cases of prenatal diagnosis. Sequence analysis for the negative cases revealed a novel mutation. Our data supports the efficiency of the Iranian Thalassemia Prevention Program

P0361. Identification of mutations, Regional Distribution and Prenatal Diagnosis of β-Thalassaemia in Iran

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We have characterized the ß-globin gene mutations in more than 1500 carriers of ß-thalassaemia and analyzed their regional distribution in the context of prenatal diagnosis program in Iran. The majority of the carriers were from Mazandaran and Gilan in the Caspian Littoral in the North, Fars and Khuzistan in the South. Utilizing the ARMS, DGGE and sequencing techniques the subjects were screened and 27 alleles were detected in the following order: IVSII-1(GàA), IVSI-5(GàC), CD36/37(-T), IVSI-1(GàA), CD8/9(+G), CD44(-C), IVSI-110(GàA), IVSI-25del, CD30(GàC), CD8(-AA), CD39(CàT), IVSII-745(CàG) , IVSI-6(TàC), CD37/38/39(-GACCCAG),
CD22/24(-AAGTTGG), CD22(GàT), CD5(-CT), CD25/26(+T), CD15(Gà), CD82/83(-G), IVSI-130, IVSII-850(GàT), -101(CàT), IVSI-128(TàG), -88(CàT) , and new mutations -26(AàC) and -71(CàT). This study shows that the underlying genetic determinants for β-thalassaemia is heterogeneous and varies in different regions of Iran. As far as prenatal diagnosis was concerned, fetal diagnosis was made for 700 pregnancies. In those cases where parental mutations could not be identified by ARMS, utilizing RFLP analysis the fetal status was inferred. As a result of this combined approach, PND was made possible in 95% of the cases.

P0362. Molecular analysis of beta-thalassaemia patients in a high incidence area of Southern Italy.

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The prevalence of 8 mutations in 84 patients with β -thalassaemia major and in 16 subjects with thalassaemia intermedia has been investigated. All the patients were Italian originating from Eastern Sicily and Calabria. Genomic DNA was amplified by polymerase chain reaction (PCR). DNA molecular investigations were performed by allele-specific oligonucleotide (ASO) hybridization to determine the following β -thalassaemia mutations: CD39 (C-T), IVS1-110 (G-A), IVS1-6 (T-C), IVS1-1 (G-A), IVS2-745 (C-G), IVS2-1 (G-A), -87 (C-G), CD6 A (-A) .
In thalassemia intermedia two mutations were statistically prevalent: IVS 1-6 T→ C (P<0.001) and CD 6-A (P< 0.05). CD 39 was statistically prevalent in β -thalassaemia major patients (P<0.01). The difference between the two groups was not statistically significant for all the other mutations. Five different genotypes were recorded among thalassaemia intermedia and 15 among β -thalassaemia major patients. Twenty-five percent of intermediate and 4.5% of major patients had homozygosity for mild mutations (group I); 62.5% of intermediate and 26.2% of major patients had combinations of mild/severe mutations (group II). Moreover, homozygosity or double heterozygosity for severe mutations (group III) in 12.5% of intermediate and 69% of major patients was found. Some genotypes were restricted to thalassaemia intermedia, including heterozygosity -87/IVS 1-6 and IVS 1-6/CD 6-A. It is essential to understand the distribution and frequency of the relevant mutations in each population where β -thalassaemias exist. This aspect is of particular importance for the correlation genotype-phenotype and for the carrier detection, genetic counselling and prenatal diagnosis.

P0363. Strategies for preimplantation genetic diagnosis for single-gene disorders

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Since 2002 have we offered PGD tests for single-gene disorders on-demand when server dominant and recessive diseases are present in the couple's family. To fulfil this offer we have standardise procedures in the PGD lab, especially concerning interpretation and verification of results to ensure a high rate of diagnosed embryos and a low rate of misdiagnosed embryos. All PGD tests were couple specific, i.e. couples were informative for at least one either linked or unlinked marker. When a linked marker was used the haplotypes of the linked marker together with the disease specific mutation or together with a flanking marker were determined. The markers were microsatellites. Single-cell amplification efficiency were assessed on single lymphocytes by measuring allele drop-out (ADO). A test set-up was only used if the ADO was ≤ 10%. All reagents used during cell biopsy, cell lysis, and PCR amplification were checked by testing for contaminating DNA. The contamination rate of the reagent should be ≤ 1 in 96 blanks to be used in a PGD test.
The diagnostic strategies for various PGD tests including Familial Adenomatous Colon Polyposis, Von Hippel-Lindau disease and Multiple Endocrine Neoplasia 1 and the outcome of PGD treatments including diagnoses efficiency, ADO rate on blastomeres, rate of misdiagnosis and pregnancy rate will be presented.

P0364. Prenatal diagnosis of 47,XX,t(2;18)(p21-q23),+18 due to maternal balanced translocation of t(2;18)(p21-q23)

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The chromosome abnormalities due to non-disjunction of maternal chromosomes during meiosis are associated with maternal age, with a sharp increase in the slope of the trisomy-age curve between the ages of 30 and 40 years. For detecting the chromosomal abnormalities during pregnancy, first and second trimester screening and diagnostic tests including triple tests, nuchal translucency, amniocentesis, corionic villus biopsies, cordocentesis are available according to the gestational age. Invasive prenatal diagnostic tests have a risk of miscarriage and therefore this procedure has been used only for women who have been identified as having a raised risk of chromosome abnormality. In this case that we represent, as the estimated risk for trisomy 18 was 1/90 according to the screening test. Because of the high risk in trisomy screening amniocentesis was performed at the 18th week of gestation. Karyotype analysis showed 47,XY, del(2)(p21-pter),+marker in the fetus. Maternal cytogenetic analysis showed a karyotype of 46,XX,t(2;18)(p21-q23). Because of the 3:1 missegragation during the meiosis in gamets of resiprokal translocation carriers, the fetuses may have a trisomic pattern. The fetus presented had an unbalanced karyotype of 47,XX,t(2;18)(p21-q23),+18 because of 3:1 missegregation during maternel meiosis. After genetic counseling the family chose therapeutic abortion.
We present this case because of its rarity as up to date no case has been reported having an unbalanced karyotype found in our case due to the parental balanced reciprocal translocation of t(2;18)(p21-q23)

P0365. Automatic detection, selection and capture of fetal cells in maternal blood by the combination of MetaferP and LMPC.

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Fetal cells in maternal blood (FCMB) are still representing a future aspect of prenatal diagnosis. Even if the analysis of FCMB never will replace invasive prenatal diagnostic procedures like chorionic villus biopsy or amniocentesis, it might be useful for e.g. validation of false positives associated with current non-invasive screening methods. In a collaboration of the University of Basel and PALM Microlaser Technologies single labeled fetal cells from maternal blood were automatically detected, selected and captured. The application of a scanning software combined with a laser mediated collection of single

cells allowed fast and reliable access to the selected cells.
The MetaferP software is capable of fast and automatic detection of specifically labeled rare cells. Data of single fetal cells, assessed as positive, were transferred to the PALM software. Pure retrievement of those cells was then performed by non-contact Laser Microdissection and Pressure Catapulting (LMPC).
This kind of investigation allows for detection, selection and capture of every cell type which might be an interesting object in non-invasive prenatal diagnosis. Even for the cultivation of fetal cells, if desired, the LMPC technology will bear good prospects. Collection of single live cells allow to grow real single cells without the necessity to apply techniques of selective stimulation of fetal cell growth.
Application of the automated scanning software MetaferP combined with the LMPC technology to capture single cells have promise to a big step forward to develop protocols for non-invasive prenatal diagnosis.

P0366. Preimplantation Genetic Diagnosis (PGD) for a male carrier of a Y;autosome translocation resulting in an ongoing clinical pregnancy.

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Males with balanced Y;autosome translocations usually present with infertility associated with azoospermia/oligozoospermia and/or abnormalities of sperm morphology. Sperm-FISH studies on carriers have reported a high prevalence of chromosomally abnormal sperm; assisted conception using intracytoplasmic sperm injection (ICSI) may therefore be problematic. We report the use of PGD using a five-colour FISH approach for a male with a balanced Y;13 translocation (46,X,t(Y;13)(q11.1;q21.3)) and severe oligoasthenoteratospermia. Routine stimulation protocols were followed by ICSI treatment; 15 eggs were collected and 9 were injected, of which 4 had 2 pronuclei. Three embryos were suitable for biopsy on day 3; a single blastomere was taken from each embryo and the nuclei hybridized with probes for the chromosome 13 long arm subtelomere region, the RB-1 locus in band 13q14, and the centromeres of the X and the Y chromosomes, differentially labelled with Spectrum Green, Spectrum Aqua, Texas Red and biotin. The biotinylated probe was detected using Cy5-streptavidin and the nuclei were counterstained with DAPI. The nuclei from two of the embryos showed abnormal signal patterns, while the third nucleus showed a signal pattern consistent with a carrier male embryo. This embryo was transferred and resulted in an ongoing clinical pregnancy (currently 32 weeks gestation), with delivery expected in April 2004. No abnormality was detected on fetal ultrasound; invasive prenatal diagnosis was declined. We are not aware of any previously reported clinical pregnancies following PGD for Y;autosome translocations. This approach provides a realistic treatment option for Y;autosome translocation carriers with or without infertility.

P0367. QF-PCR: a Rapid Prenatal Detection of Common Chromosome Aneuploidies.

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Karyotype analysis from cultured cells needs, on average, 11-14 days for their results while autosomal trisomies, which account for about 80% of significant abnormalities, can be detected within 24-48 hours by quantitative fluorescence (QF) PCR. Most of these abnormalities are primary trisomies for chromosome 13, 18, 21, or numerical sex chromosome abnormalities (1). We have used QF-PCR to assess relative allele dosage at polymorphic loci of chromosomes 13, 18, 21 and sex chromosome in 996amniotic fluid and 281 chorionic villus samples. The procedure uses a "one-tube test" in which markers are co-amplified in one multiplex PCR assay. All samples were also analysed by full karyotype on cultured cells. QF-PCR showed normal results in 1277samples (98%), while in 25 samples (1.95%) trisomy 21 was detected; 8 samples (0.6%) were trisomic for chromosome 18; 2 samples (0.15%) were trisomic for chromosome 13; 3 samples (0.23%) was diagnosed as Turner Syndrome; one (0.09%) as Klinefelter Syndrome; 2 (0.15%) were diagnosed as 69,XXX for a total of 3.2% cases with chromosome aneuploidy. No false positive results were obtained, while in three cases, due to maternal blood contamination the analysis gave ambiguous results.
Our results confirm that QF-PCR technique is a rapid testing able to diagnose chromosome aneuploidy accurately in prenatal diagnosis both on amniotic fluid and chorion villi. The use of QF-PCR as a stand-alone test for some referral indications is being considered at our institution.

P0368. The effect of previous pregnancies and maternal transfusion on sex determination using the SRY region detection with real-time PCR in maternal plasma

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Non-invasive methods using maternal plasma for molecular genetic diagnosis become an important field of interest in prenatal diagnosis. Detection of free fetal DNA in maternal plasma with real-time PCR has been shown to be useful for fetal gender determination. A few publications report that the fetal DNA can be detected in maternal plasma years after delivery. The aim of our study was to control the sensibility and specifity, of the real-time PCR amplification of the SRY region from maternal plasma. Maternal plasma before amniocentesis, and amnionic fluid samples were obtained from 54 pregnant women. Real-time PCR analysis of the SRY region was performed in order to determine the fetal sex. Routine karyotyping was also performed on the samples. We found male fetuses in 26 of 51 pregnancies by cytogenetic analysis. In 20 cases there previous child was male and in 5 cases there was transfusion in maternal history. Real time PCR of maternal plasma has been positive for the SRY region in 27 cases. In 51 cases the cytogenetic gender and the real-time PCR results were correlating. In one case of 46,XY karyotype the PCR reaction for SRY region was negative, in two cases of SRY positivity the karyotype was 46,XX. In one of the false positive cases the previous child was male. The result of our study suggest that real time PCR detrmination of fetal gender from cell free fetal DNA in maternal plasma seems to be not influenced by the gender of previous pregnancies and maternal blood transfusion.

P0369. The predictive value of 45,X detected in CVS. Our experience.

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The birth prevalence of Turner syndrome has been estimated to be from 1 in 2,000 to 1 in 5,000 female live births. Approximately 1% to 2% of all conceptuses have a 45,X chromosome constitution. The majority of these spontaneously abort, usually during the first trimester of pregnancy. However, not all the 45,X diagnoses performed on chorionic villi samples reflect the chromosome constitution of the fetus. The loss of an X or Y chromosome in placental tissue is a relatively frequent event We detected 22 cases of monosomy X among 5,896 first trimester (CVS) diagnoses (0.37%), two of them mosaic. Seven cases turned out to be confined to placenta: fetal karyotypes were 46,XX (n=4), 46,XY (n=3). Two other cases showed discrepant chromosome constitution between CVS (45,X) and fetal tissues (mos45,X/46,XX and mos 45,X/46,i(Xq)). The remaining cases reflected the fetal karyotype, since ultrasonography revealed features usually associated with Turner fetuses: increased nuchal translucency, cystic hygroma, lymphedema or hydrops fetalis. We describe the indication for study, results and follow-up of all cases. From our experience we would recommend to confirm the CVS finding of 45,X in amniocytes in those cases with normal ultrasound scan.
This work has been partially supported by the „Red de Centros de Genética Clínica y Molecular" (V-2003-Red-07-O)

P0370. Utilisation of PAPP-A / PROMBP-complex in early screening of impaired prenatal development and detection of different types of acute coronary artery disease.

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Efficiency of PAPP-A / PROMBP and beta-hCG maternal serum screening was evaluated by degree of deviation from norm (1,6-1,9 MoM). This complex was also studied in patients with stable-/unstable angina pectoris and with acute myocardial infarction, versus controls. Delphia and Kryptor technologies were used in 1,494 women during the 11.-14. weeks of gestation. Analyte serum levels were adjusted according to weight and gestational age. Prenatal screening efficiency was evaluated by proportion of pregnancies with spontaneous abortions, prematurity and preeclampsia (PD) and/or severe anomalies and aneuploidies (CA). No severe chromosomal anomalies were found in 570 controls (>0.6 - <1.9 MoM). In pregnancies with 0.5-0.6 and 1.9-2.0 MoM 45,X/46,XX (1/127) was disclosed. Trisomy 21 and FraX-A syndromes were detected in 1/414 cases with one analyte < 0.5 or > 2.0 MoM. Triploidy (1x), trisomy 13 (3x), trisomy 21 (2x), 45,X/46,XX (1x) were discovered in 7 / 83 (8.4 %) pregnancies with both abnormal analytes. That is significantly higher than in controls ($p < 0.001$) and than the detection rate of trisomy 21 from II. trimester screening (2/400; 0.5%; $p < 0.001$). The risk of CA was not increased in this group contrary to the increased risk PD (2 / 83; 2.4 %; $p < 0.001$). The patients with unstable angina pectoris and acute myocardial infarction have highly significantly increased levels of PAPP-A / PROMBP compared to healthy individuals and patients with stable coronary artery disease. Specificity and sensitivity was significantly higher than in troponin I and CRP. *Supported by ICA2-CT-2000-10012, MSMT-111300003, MZCR-00000064203*

P0371. Prenatal Diagnosis and Deletion Screening of Duchenne Muscular Dystrophy in Iranian Families

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Duchenne muscular dystrophy (DMD) is one of the most common X-linked genetic disorders, which is caused by mutations in Dystrophin gene and manifests with severe muscle weakness and eventually leads to death in the second or third decades of life. In the absence of an appropriate cure, prenatal diagnosis appears to be the best approach to reduce the burden of the disease on the individual family and ultimately on the society.
During the last five years, prenatal diagnosis was offered on request to approximately 85 families, having one or more affected male children. Initially, the deletions in the DMD gene were identified by Multiplex PCR, screening for 20 exons. Then, in all patients, three intragenic RFLPs and two main CA repeats that have been shown to be highly heterozygous in the previous studies, were used to perform carrier detection and linkage analysis.
Deletions were found in 43 affected boys (50.5%).In 47 families the intragenic RFLPs were utilized
and in 26 of them one or more RFLPs were informative
(55%). In 30 families two microsatellite repeat analysis were done to identify the mutant alleles and in 12 families, 5'-Dys was informative. However, in Iranian families, CA repeats 3'- DYS analysis does not have any significant heterogeneity.
Prenatal diagnosis was performed in 25 families.
Therefore, it is concluded that multiplex PCR technology and three intragenic RFLPs, can be used effectively for PND and carrier detection. New systems such as short tandem repeats (STRs) are being used to further improve DMD prenatal diagnosis.

P0372. Autosomal Recessive Type SCID and Prenatal Diagnosis: Case Report

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Severe Combine Immune Deficiency (SCID), consists of group of genetic disorders characterized by recurrent infections due to deficiency in T cell, B cell or both lymphocyte groups. Bone marrow transplantation is the only way to cure the disease. Because of the severe problems encountered during bone marrow transplantation, prenatal diagnosis is crucial.
A 32-year old pregnant mother was referred to us at the 17th week of the gestation. The parents were first cousins. The family had 3 healthy children and a child with SCID. Two previous children (5-month old boy, 3-month old girl) of the family died from SCID. The 5-year old SCID child had bone marrow transplantation at the age of 1. Until their referral to our center, no molecular tests have been performed. Because of the advanced gestational age and the absence of molecular analysis, an alternative diagnosis was planned for prenatal diagnosis.
Cordocentesis was performed at the 20-21th week of gestation and T and B lymphocyte levels were assessed. Lymphocyte culture and fetal karyotype were performed in order to understand whether there were lymphocytes circulating. HLA typing was performed to differentiate maternal and fetal blood. In fetal blood 46% of CD3, 4% CD19, 35% CD4, 20% CD8 , 1% CD3+ HLA DR were detected . Fetal karyotype was 46,XY. Maternal fetal contamination was excluded by HLA typing. Regarding the lymphocytes distribution according to their immunological markers and the availability of fetal karyotype from lymphocyte culture, the fetus was considered to be normal.

P0373. Prenatal diagnosis of de nova unbalanced translocation 8p;21q using subtelomeric probes

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We report an unbalanced translocation involving chromosomes 8 and 21 in a fetus showing ultrasonographic abnormalities at the second trimester of pregnancy. A 41 years old pregnant women, gravida 1 para 0, was referred to genetics subdivision at the 16th week of gestation because of advanced maternal age and fetal pelvicaliectasis on ultrasographic exmination. Pregnancy had occured following ICSI threatment. After genetic counseling amniocentesis was performed. Fetal karyotype analysis revealed a karyotype of 46,XY,8p+. Ulrasonographic examination repeated at the 20thweek of gestation showed intrauterin growth retardation, ventriculomegali, hypoplasic corpus callosum, cerebellar structural abnormality and pelvicaliectasis. Both parents had normal chromosomal composition. Molecular cytogenetic studies (FISH) using chromosome-specific subtelomere probes showed a terminal deletion of 8p and trisomy of 21q subtelomeric region . Further analysis with Down Syndrome specific region probes revealed two signals. The couple decided to terminate the pregnancy. This is the first prenatally diagnosed case showing t(8p;21q) and born to parents having normal karyotaypes.

P0374. Prospective evaluation of first trimester risk screening in a single center

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Close to 5000 consecutive pregnancies have been screened during a six years period. Risk screening was offered after appropriate counseling to all patients in the outpatient clinic and those refered for specialized ultrasound services with a fetal crown rump length between 45 and 86 mm (i.e. gestational ages 11+3 to 13+6 weeks). Individual risks were assessed based on nuchal translucency (NT) measurements and maternal age in all patients as well as maternal serum markers (free ß hCG, PAPP A) in a subset of patients using the software of the Fetal Medicine Foundation, London. This institution audits the program in regular intervals.
As of Feb 9, 2004 4730 consecutive pregnancies have been screened. A total of 69 (1,5 %) unbalanced chromosomal abnormalities including 36 cases of trisomy 21 were diagnosed in the study group. The detection rate of risk screening based on NT and maternal age using a cut-off of 1:300 was 83 % for trisomy 21. It did not differ for all unbalanced chromosome anomalies. A risk of 1 in 300 or higher was calculated in a total of 531 pregnancies giving a false positive rate of about 10 %.

These results are in good accordance with published data and clearly demonstrate the advantages of first trimester risk screening as compared to traditional approaches still offered in a large number of pregnancies. The decreasing number of invasive procedures observed in our center and others appears to be a consequence of the superior performance of first trimester risk screening.

P0375. Diagnostic problems in cytogenetic examinations of prenatally detected cases.

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We present results of cytogenetic examinations in 5 cases of chromosomal rearrangements in prenatal diagnosis which were difficult or impossible to diagnose using routine cytogenetic banding techniques. FISH was applied to identify these rearrangements..
In case 1 we found small additional fragment on the long arm of the chromosome 13. After using painting probes this fragment appeared to be a part of chromosome 21 and it is of paternal origin. Applying 21q22 specific probe we excluded trisomy of this region. In this case we could not establish whether the karyotype of the fetus was balanced or weather the father had balanced translocation 13;21. In case 2 of de novo translocation 6; 12 we used subtelomeric probes and we found that a break point of chromosome 6 was located distal to used probes (using commercially available probes we could not establish whether the translocation was balanced).
In case 3 FISH with subtelomeric probes was applied to find out whether paternally inherited translocation 1;17 was balanced. The break point of chromosome 17 was close to the subtelomeric region.
In case 4 USG and echocardiography findings could indicate 22q11.2 syndrome which was confirmed using TUPLE 1 probe.
In case 5 cytogenetic examination was performed because of abnormal USG. With routine methods the karyotype seemed to be normal. However, phenotype of the newborn suggested Wolf-Hirschorn Syndrome and it was confirmed with specific FISH probes.
FISH is a very important but sometimes insufficient tool in solving cytogenetic problems, particularly in prenatal diagnosis.

P0376. The Frequency of Human Papillomavirus Infection in Iranian Patients with Cervical Cancer

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Human papiloma virus (HPV) is a well-known etiologic factor of cervical cancer in Western countries. However, despite a relatively high prevalence of cervical cancer in Iran, no studies are available on the relationship between HPV infection and cervical cancer. Of ninety plus types of HPV, HPV-16 is the most prevalent in cervical cancer, followed by HPV-18. A retrospective study was performed on 107 cervical cancer paraffin-embedded histopathology blocks. DNA extraction was performed through standard procedures followed by Multiplex Polymerase Chain Reaction amplification with two pairs of primers (one as internal control) which were designed to detect HPV in the related tissues. The products were run on 8% acryl amide gel. HPV typing was done for the types 16 and 18 positive controls of which were available in Iran. We found 78 out of 107 cases with pathological indecices of cervical cancer were positive for HPV. In our study nearly 70% of the malignant cervical lesions were contaminated with HPV. This finding confirms the previous reports on the significant association of HPV with cervical cancer.

P0377. The detection of 11q23 and 13q14 deletions in patients with chronic lymphocytic leukemia by fluorescence in situ hybridisation method

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Chronic lymphocytic leukemia (CLL), is the most common leukemia characterized by the accumulation of mostly CD5+ mature-appearing B lymphocytes in humans.
The data about the genomic aberrations occuring in CLL has improved recently and will undoubtedly provide important clues to the clinical behaviour of the disease and new targets for effective therapy. FISH made it possible to identify chromosome aberrations in approximately 80% of CLL cases by using locus spesific probes. Deletions are the most frequent structural chromosome abnormalities in CLL and 13q14 and 11q22-23 are commonly deleted regions.
Del (13)(q14) is considered to be the predictor of good prognosis whereas del (11)(q23) is the predictor of poor prognosis.
The aim of our investigation is to evaluate the significance of 11q23 and 13q14 deletions in CLL patients and to correlate our laboratory findings with clinico-biologic characteristics, for the first time in Turkey.
40 patients with CLL were included in our study. We detected deletions in 70% of the patients. Fifteen out of 40 (37.5%) patients were found to have del (11)(q23) and 13 of them (32.5%) were found to have del (13)(q14). Twelve patients (30%) had none of the deletions and none of the patients had both. We compared these results with other prognostic factors but no statistically significant correlation was established.
We think that more patients must be included into the study and the deletion analysis should be performed at different times during the progression of the disease to have an idea about the prognosis.

P0378. Genetic polymorphisms of biotransformation enzymes in patients with hematological malignances

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Polymorphisms in genes encoding carcinogen-metabolizing enzymes may have relevance in determining susceptibility to cancer. Individuals carrying the more active form of enzyme involved in the activation of carcinogen, or less efficient alleles of detoxifying enzymes, will be at great risk of cancer.
PCR-RFLP-based genotyping assays were used to determine the frequency of polymorphisms in CYP1A1, CYP2E1, mEPHX, NAT2, GSTM1, GSTP1, GSTT1, MDR1 genes in a case-control study comprised 120 children patients with hematological malignancies and 167 healthy individuals from Bashkortostan republic.
We found statistical significant association of heterozygous genotype of CYP1A1in the patient group p=0,01; OR=3,75). The combination of normal alleles CYP1A1/CYP2E1 genes was higher in the group of healthy individuals (p=0,04; OR=0,44). We established that fast mEPHX phenotype, assessed by genotyping, was higher in the whole patient group (0,22 versus 0,14), but normal mEPHX phenotype was higher in the non-Hodgkin*s lymphoma (NHL) patient group. We determined more frequent genotype combinations of NAT2 gene in patient groups (acute lymphoblastic leukemia - NAT2*5/6, NAT2*5/4; acute myeloblastic leukemia (AML) -NAT2*5/5, NHL - NAT2*5/4). The frequency of GSTT1 gene deletion was decreased in the patients group (p=0,03; OR=0,47), there was not anyone with GSTT1 gene deletion among patients with AML. We found increased frequency of GSTM1 gene deletion - 0,64 and decreased frequency of MDR1 gene mutation - 0,36 in the group of AML patient (versus 0,40 and 0,49 in controls, correspondingly).
Our findings seem to suggest an influence of genetic polymorphisms of xenobiotic metabolizing enzymes on the susceptibility to hematological malignances.

P0379. The SSCP profiles of p53 gene in 3-methylcholanthrene and butylated hydroxytoluene induced rat lung tissues

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SUMMARY

In the present study it was aimed to find out the mutagenic effects of 3-Methylcholanthrene (MCA) and agent Butylated hydoxytoluene (BHT) on rat lung tissues. A group of rats (*Rattus norvegicus wistar albino*) were given one cancer induced agent once a week with a MCA 40mg/kg for six weeks; the second group of rats were given antioxydant BHT 200mg/kg once a week again for six weeks. For the other dual experimental group rats were given a single dose of MCA 40mg/kg and then BHT 200mg/kg intraperitonally for six weeks. After 26 weeks all rats were killed and lung tissues were taken for genomic DNA isolation. For the mutation analysis of exon-5 of p53 gene the PCR-SSCP based methodolgy was used in the present study. On the other hand rat tissues were evaluated radiological and scintigraphically for possible tumoral lesions.

According to our experimental results; no tumoral appearence was diagnosed in control and all experimental group rats tissues histopathologicaly. In group one due to high dose administration of 3-MCA, malign sarcoma were observed(55.55%) in the around of injection areas. Mutations were determined in exon 5 of p53 gene (11.11%) in experimental group one. In lung specimens of group two, high rate of(42.85%) p53 exon-5 mutation (which usually under goes mutations in cancer cases) were also determined. No tumor or mutations were detected in control and experimental group three.

P0380. Proto-oncogene c-myc SSCP profiles in human bladder urothelial carcinoma tissues

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SUMMARY

Although the process of bladder tumorigenesis requires multiple genetic events, it is unclear to what extent carcinogenesis proceeds through preferred secondary pathways following a specific initiating oncogenic event. We studied the expression and mutation analysis of the human c-myc proto- oncogene in human bladder urothelial carcinoma tissues by both immunohistochemistry and PCR-SSCP analysis, respectively. The immunohistochemical findings were showed that the c-myc proto-oncogene is primarily expressed in cells of both invasive and non-invasiv urothelial carcinomas that have a different histological grades and the exon-2 of c-myc gene was found to be mutated in one case which is in advanced stage of bladder cancer.

P0381. A genome-wide scan for linkage disequilibrium with breast cancer in an eastern Finnish population

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Breast cancer is the most common of cancers among women in industrialised countries. Many of breast cancer risk factors are known but the majority of the genetic background is still unknown. Linkage disequilibrium based association is a powerful tool for mapping disease genes and is suitable for mapping complex traits in founder populations. Our aim was to find new genetic medium or low-penetrance breast cancer risk factors in an eastern Finnish population by identifying chromosomal regions that are associated with breast cancer. Our case-control set is from the province of Northern Savo in the late-settlement area of eastern Finland. This population is relatively young and genetically homogeneous. We screened the autosomes with 366 polymorphic microsatellite markers in a set of 49 breast cancer cases and 50 controls and further analysed 69 markers flanking significant loci and calculated differences between frequencies of the estimated two, three and four-marker haplotypes in case and control groups. Our results suggest four breast cancer associated regions in three chromosomes in an eastern Finnish population.

P0382. Methylation inactivates expression of CDP-diacylglycerol synthase 1 (CDS1) in hepatocellular carcinoma

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CDS1 is an enzyme required for the regeneration of the signaling molecular phosphatidylinositol-4,5-bisphosphate (PIP2) from phosphatidic acid. These phosphoinositides and their cleavage products are a class of second messengers, which are involved in cell growth and oncogenesis. We examined the expression of *CDS1* in 52 hepatocellular carcinoma and paired non-cancerous tissues by real-time quantitative reverse transcription-polymerase chain reaction analysis. The results showed that the expression levels of *CDS1* significantly decreased in hepatocellular carcinoma. Mutational analysis of the whole gene and methylation analysis of cytosine-phosphate guanosine (CpG) sites at the promoter area were further performed to investigate the possible mechanisms. However, no mutation was found within the coding region. Interestingly, in the promoter area of *CDS1* gene, most of the CpG sites were methylated in 73% of the cancerous tissues; in contrast, only a partial methylation of CpG was found in 50% of the non-cancerous tissues. Our results suggest that the down regulated *CDS1* expression in hepatocellular carcinoma was due to the inactivation of the *CDS1* gene by methylation and that the differential expression correlated to the ratio of CpG sites being methylated. Therefore, methylation of *CDS1* may play a role in the oncogenesis of hepatocellular carcinoma.

P0383. Identification of two putative telomerase suppressor gene loci on chromosome 4 involved in cervical carcinogenesis

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Telomerase activity is detectable in most human cancers and immortal cell lines. In contrast, in normal diploid human cells progressive reduction of telomere lenght to critically short sizes has been correlated with the cessation of cell division and the onset of senescence. Previously we have provided evidence for the localization of a telomerase suppressor gene on chromosome 4. The aim of our study is to determine a region on chromosome 4 which is associated with telomerase suppression.

Microcell-mediated chromosome transfer was performed to introduce a single copy of the entire human chromosome 4 and different derivative chromosome 4 into HPK II cells. Cell lysates were prepared directly from individual hybrid cell colonies (100-300 cells). Telomerase activity was determined using a telomerase PCR ELISA kit.

Strong suppression of telomerase activity was only found in a subset of HPK II hybrids in which chromosome 4 or three of the eight der(4) chromosomes had been introduced. Our data suggest that two putative telomerase suppressor gene loci map to the long arm of chromosome 4 ((4p13-q13.2 and 4q25-q31.21).

Currently, these loci will being defined more precisely by matrix CGH-analyses.

P0384. Prostate specific antigen (PSA) in patients with prostate cancer or benign prostate hyperplasia(BPH): The first molecular investigation report from Iran.

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Background: Prostate cancer is the second commonest form of cacer in men. Detection of circulating PSA transcripts has effectively been used for early diagnosis of prostate cancer cells.
Objective: This investigation employed a reverse transcriptase polymerase chain reaction (RT-PCR) technique to distinguish the patients with either localized or metastatic prostate cancer (PCa) vs. BPH and control subjects, as compared with clinical and pathological records.
Methods: With reservation of ethical issues, blood samples were collected from 60 cases. Based on pathological and clinical findings, 29 patients (24 with localized cancer, 5 with metastases), 18 with benign BPH, and 13 healthy (including 3 females) subjects as negative controls, were selected from Shariaty, Mehrad, Sina,, Khatam and Atye hospitals in Tehran, Iran. An RT-PCR for a 260bp PSA transcript was then performed. Clinical and pathological records were used for the assessment and comparison of PSA RT-PCR results.
Results: None of the control subjects and BPH (with 4 exceptions) were found positive by RT-PCR (Specificity=72.7%). In patients with localized prostate cancer, 21 out of 24 were found PSA positive (Sensitivity=83.4%) and the remaining 3 have showed PSA negative (Positive predictive value=80%). All of 5 metastases patients (100%) revealed PSA positive results.
Conclusions: Our data reflects the clinical relevance and significance of RT-PCR results as assessed with clinical and pathological examinations. PSA RT-PCR might be used as a powerful means for diagnosis, even when either pathological or clinical findings are negative, and could be employed for further molecular epidemiology surveys.

P0385. *PTEN* mutations in African and Caucasian patients with endometrial cancer.

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PTEN/MMAC1/TEP1, a tumour suppressor gene on chromosome 10q23, has been implicated in the development and/or progression of a wide range of cancers, when perturbed. PTEN, a lipid- and protein phosphatase, negatively regulates cell growth, migration and invasion via the FAK-, MAPK- and PI3K- pathways. *PTEN* is most commonly mutated in all stages of endometrioid adenocarcinoma of the endometrium (EEC) with a frequency of 26 % to 52 %. In previous studies, differences in the *PTEN* mutation frequency between African and Caucasian women were observed, suggesting a possible dissimilarity in the molecular pathogenesis of endometrial cancer in these groups.
Paraffin-embedded tissue samples from South African patients with EEC [n=37] were analysed with PCR-SSCP. Aberrant migrating samples were sequenced. Sixty % (22/37) of the cancers had somatic mutations. Alterations were located throughout the gene with the exception of exons 2, 4 and 9. These changes include 10 frameshifts, 6 nonsense, 6 missense and 3 splice site mutations, 1 in-frame deletion and 4 polymorphisms. Codon 130 in exon 5, which lies within the phosphatase core motif of PTEN, is a mutational hot spot limited to the African patients. Mutations occurred in 56 % (14/25) African, 64 % (7/11) Caucasian and 100 % (1/1) Indian patients. No ethnic disparity was observed between the African and Caucasian patients.

P0386. Interphase FISH and Spectral Karyotyping analysis in B-CLL young patients

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B-cell chronic lymphocytic leukaemia (B-CLL) is the most common form of adult leukaemia in the Western countries, characterized by accumulation in bone marrow and peripheral blood of mature lymphocytes. Little is known about the genetic alterations underlying the pathogenesis of this disease, mainly because cytogenetic studies are hindered by the low mitotic index of tumour cells and the limited resolution of chromosome banding. Bone marrow samples of 50 B-CLL patients were analysed by Interphase FISH (I-FISH) for the regions 13q14, 11q22-23, 17p13, 14 q32 and 12p11.1-q11.1. Fifteen of the patients were also studied by Spectral Karyotyping (SKY), to look for correlation between cytogenetic abnormalities and clinical features.
Statistical correlations were evidenced by I-FISH between 11q23, 13q14 and 14q32 deletions and patients' clinical characteristics: 14q32 deletion showed a significant association with the presentation age (p=0.008); while 11q23 deletion was associated with the presence of lymphoadenopathy (p=0.04), the need of chemotherapeutic treatment (p=0.0002) and a shorter treatment free interval (p<0.0001). Interestingly, the presence of 11q23 deletion was found to predict rapid disease progression even in the patients with low-risk stage at diagnosis. Also the presence of 13q14 deletion is associated with a shorter treatment free interval (p=0.0003).
SKY analysis did not evidence numerical or structural changes, including those monitored by I-FISH, in the metaphases available from a subset of patients. We conclude that I-FISH is a powerful approach to detect genomic aberrations in diseases such as B-CLL where abnormal clones may escape detection because of the low number of dividing cells.

P0387. Identification of an Alu-mediated 5.1 kb deletion removing *BRCA1* Exon 17 in a German Breast and Ovarian Cancer Family

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During the last years several methods have been developed revealing a significant proportion of *BRCA1* exon deletions or duplications in breast/ovarian cancer families with high probability of *BRCA1/2*-linked predisposition, which had been tested negative by standard screening methods. Comprehensive data about the spectrum and frequency of large genomic rearrangements of the *BRCA1* gene in the German population are still missing. Establishing a multiplex PCR of short fluorescent fragments enabled screening a collective of 32 breast/ovarian cancer families, previously tested negative for *BRCA1/2* mutations by standard sequencing. This method allows a sensitive and specific evaluation of the 22 coding exons and a fragment from the 5' untranslated region including the promoter α. Two deletions (exon 17 and exon 2) could be found and confirmed by the MLPA technique. This corresponds to a frequency of 3.5% (2/58 tested individuals with BRCA1 mutations), which is lower than the frequency reported from other populations. The rearrangement affecting exon 17 was proved by long range PCR, and sequence analysis of the mutant allele revealed a 5.1 kb deletion. This deletion is the result of a recombination between two closely related Alu sequences and is the third one in the genomic region surrounding exon 17. These data show that Alu-mediated recombination seems to be an important mechanism leading to pathological inactivation of the *BRCA1* gene, and that this region might be a site especially prone to this type of rearrangement although the density of Alu sequences is not higher than in other areas of the gene.

P0388. P53 gene mutations in primer tumor tissues and surgical margins of patients with squamous cell carcinoma of the head and neck

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Head and neck cancers are involved in the six most common cancer in the world. Tumors of these type cancer which is closely related with etiological factors such as smoking and alcohol habits, have mutations in p53 gene as frequently as 50-60%. Furthermore, because almost 10-30% of patients with head and neck cancer develop local recurrences, it is necessary that surgical margins should be evaluated with sensitive molecular genetic methods. In this study, we examined p53 gene mutations on tumor samples and surgical margins of 34 patients with head and neck squamous cell carcinoma localized larynx by heterodublex analysis (HDA) and sequencing. P53 mutations were determined at totally 15 patients (44.12%) involved of 3 patients (8.82%) were at codon 175addAT, 1 patient (2.94%) was at codon 175delGC, 1 patient (2.94%) was at codon 206G→A, and 11 patients (32.35%) were codon 248delC. Moreover, we determined same mutations at the surgical margins of 5 out of 15 patients (33.33%) which have a p53 mutation. As a results of present study, we belive that p53 gene mutations have an important role for formation of squamous cell carcinoma of head and neck, and they play an important role at the increasing the rate of aggressive of tumor. Moreover, the histopathologically negative surgical margins may be had a p53 mutations, but the follow up period is necessary to be prolonged for understand whether p53 mutations in surgical margins are effected to survival rate or not.

P0389. Determination of Germline BRCA1 gene mutations in Turkish breast and breast/ovarian cancer families

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Breast cancer is one of the most common malignancies affecting women in all the world. The first major breast and ovarian cancer predisposition gene, BRCA1, was identified in 1994 by Miki et al. This gene is a putative tumor suppressor gene located on chromosome 17q21 that has been implicated in DNA repair recognation. BRCA1 gene mutations are responsible for tumors in 40-50% familial breast cancers and in 80-90% of breast/ovarian cancers. There is little knowledge about the contribution of BRCA1 gene mutations to breast and breast/ ovarian cancers in the Turkish population. In the present study, we screened to BRCA1 gene of 92 individuals from 39 families with breast and breast/ovarian cancer by heteroduplex analysis (HDA). As a result of DNA sequencing of HDA positive samples, four different variants in BRCA1 were identified in six (15.4 %) families. Three of these variants were novel mutations such as intronic 5396+36 C→G;5396+38 C→A ,and missense 3663 C→A. Fourth variant was 5382insC mutation observed in specifically Ashkenazi and Russian population. Missense 3663 C→A mutation was observed in four families (%10.3). Intronic 5396+36 C→G; 5396+38 C→A variants , and frameshift 5382insC mutation were determined only one each family (%5.1). Our results revealed that the mutations were clustured with in exon 11 of BRCA1 gene in Turkish population.

P0390. The Sdhb Gene Is Non-implicated In Neuroblastoma

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Introduction Neuroblastoma and pheochromocytoma have the same embrional origin. They are originated from neural crest cells, and usually affects suprarenal glands. The SDHB gene encodes to the B subunit of succinate dehydrogenase, a protein implied in the electronic transport chain and Krebs cycle. Some mutations have been described in this gene in pheochromocytoma, and because of its localization in 1p35-36 this gene could be an appropiate candidate for its study in neuroblastoma.

Aims The aim of this study was to analyse neuroblastoma tumors in order to assess a possible implication of this gene in neuroblastoma development.

Patients and Methods We have studied 29 neuroblastoma tumor samples from different stages. Mutation search in genomic DNA was carried out after individual amplification of each one of the 8 SDHB exons by SSCP analysis and following sequenciation of those samples with migration pattern variants.

Results We have found no variant except for a polymorphism in two neuroblastoma samples. The polymorphism was an A®C in third position of codon 6 (exon 1) in heterozigosis, which implies no aminoacid change in the SDHB subunit.

Conclusion The SDHB gene, a positional candidate gene, is unlikely related with the initiation or tumoral progression on neuroblastoma.

P0391. The diffuse induction of *NDRG1* gene expression in human cancers

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In vitro experiments showed that the RNA of *NDRG1* gene is induced under conditions of severe hypoxia, nickel exposure or prolonged elevations of intracellular calcium. Ndrg1 protein is expressed at low levels in normal human tissues whereas overexpressed in a variety of cancers, including lung, brain, skin, liver, prostate, breast, and kidney cancers. The mechanism of *NDRG1* overexpression in cancer cells involves a state of hypoxia which develops at initial stages of tumor progression, and Ndrg1 protein becomes a signature for this hypoxic state. The induction of *NDRG1* by hypoxia is mostly dependent on HIF-1 transcription factor. However, induction of the gene by long term hypoxia involves some other HIF-1 independent pathways, namely Ca^{2+} mediated c-Jun/AP-1 and PI3-K pathways. Despite of being the major regulator of hypoxia response, HIF-1 transcription factor is a very unstable protein which is rapidly degraded, and therefore itself is not a good tumor marker. In contrast, its downstream products Ndrg1 protein and mRNA are extremely stable. The low level of Ndrg1 expression in normal tissues compared to their cancerous counterparts combined with the high stability of the protein and its RNA makes this gene a new, valuable cancer marker. Therefore, Ndrg1 could be used in early diagnosis of human cancers. Moreover, its strong induction by hypoxia and calcium suggests that Ndrg1 protein could play a pivotal role for cancer cell survival and proliferation. Hence, it may also be possible to direct therapy towards Ndrg1 protein with drugs that specifically disrupt the functions of this protein.

P0392. Molecular Analysis of the CALM/AF10 Fusion Transcripts in Eight Cases of Leukemia and Expression Profiling Revealing HOX Gene Deregulation

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The CALM/AF10 fusion gene, which is a result of the t(10;11)(p13;q14), is found in undifferentiated leukemia, acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and malignant lymphoma. It was reported to be the most common fusion protein in T-ALL with TCR gamma/delta rearrangement.

We have analyzed samples from eight patients with different types of leukemia: case 1 (AML), case 2 (AML M0), case 3 (pre T-ALL), case 4 (acute leukemia), case 5 and case 6 (ALL), case 7 (T-ALL), case 8 (pro T-ALL) with t(10;11) without MLL involvement. The samples were tested for the presence of the CALM/AF10 and AF10/CALM fusion mRNA by RT-PCR and sequence analysis. We found two different breakpoints in CALM at nucleotide 1926 and 2091 and a new exon, with 106 bases after nucleotide number 2091. In AF10 three breakpoints were identified: at position 424, 883 and 979. In five patients it was also possible to amplify the reciprocal AF10/CALM fusion transcript. There was no corrrelation between disease phenotype and breakpoint location. Expression profiling using Affymetrix technology was performed in 5 cases. Preliminary analysis

of microarray expression profiling revealed high expression levels of the HOX genes HOXA5, HOXA9, and HOXA10 in four cases and of the homoeobox gene MEIS1 in all cases.
The observed overexpression of HOX genes is reminiscent of the pattern seen in leukemias with rearrangements of the MLL gene, normal karyotypes and komplex aberrant karyotypes suggesting a common effector pathway (i.e. HOX gene deregulation) for these diverse leukemias.

P0393. Unraveling the genetics of Patched-related rhabdomyosarcoma and medulloblastoma

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The human homologue of the Drosophila segment polarity gene PATCHED 1 (PTCH1) is a member of the sonic hedgehog (Shh) signaling pathway, which plays an important role in development and tumorigenesis. Germline mutations in PTCH1 are found in patients with basal cell nevus syndrome, which is characterized by a high incidence of basal cell carcinoma (BCC), medulloblastoma (MB), and rhabdomyosarcoma (RMS). Somatic PTCH1 mutations have been detected in sporadic cases of BCC and MB. The significance of Ptch1 as a tumor suppressor gene is also highlighted by the phenotypic consequences of its inactivation in mice, which develop RMS and MB. These tumors consistently overexpress downstream targets of the activated Shh signaling pathway such as Gli1, Igf2 and Ptch1. In order to examine other molecular events associated with formation of these tumors, we used cDNA microarrays to define transcriptional changes in RMS and MB. Our data show that cell cycle regulators (cyclins, Cdk4, p21), cell survival factors (Igf2, Bcl-2), signal transduction proteins (members of Shh, Wnt, Jak/Stat, Tgf-beta signaling) and suppressors of metastasis (Cd63, Nme2) are transcriptionally up-regulated in Ptch1-associated tumors.
We furthermore describe that overexpression of the growth arrest and DNA-damage-inducible gene Gadd45a is common in Ptch1-associated tumors and Ptch1 null embryos. These results suggest that cDNA microarray technology is a useful tool to discover genes involved in the development of RMS and MB that arise in response to a persistent activation of Shh signaling. This approach may provide novel data for diagnosis, treatment and prevention of human PTCH1-related malignancies.

P0394. A Single Nucleotide Polymorphism in the 5' Tandem Repeat Sequences of *Thymidylate Synthase* Gene Predicts for Response to Fluorouracil-Based Chemotherapy in Advanced Colorectal Cancer Patients.

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Thymidylate synthase (TS) is a key enzyme in folate metabolism and the primary target of 5-Fuorouracil (5-FU). A repeat polymorphism in the TS promoter region (TS*2 *versus* TS*3) is associated with TS expression and with the efficacy of 5-FU-based chemotherapy in colorectal cancer (patients homozygous for TS*3 have a higher TS activity and a poorer response to 5-FU therapy than patients homozygous for TS*2). Recently, a common G>C SNP at the 12th nucleotide of the second repeat in the TS*3 alleles that alters transcriptional activity has been described. According to the combination of SNP and VNTR, three alleles can be defined: *2, *3G and *3C. The *3G allele had three to four times greater efficiency of translation that other polymorphic alleles. The allelic frequencies in Spanish individuals are as follows: 43% correponds to *2 allele; 25% to *3G and 32% to *3C, indicating that the G to C base change is a common polymorphism in this population.
TS genotypes of the 89 patients diagnosed with metastatic colorectal cancer and undergoing 5-FU-based chemotherapy were classified into high expression type (2R/3G; 3C/3G; 3G/3G) and low expression type (2R/2R; 2R/3C; 3C/3C).
The obtained results indicate that the double polymorphsim in the TS tandem repeat sequence may provide a potential for more effective fluoropyrimidine responsiveness in patients with advanced colorectal cancer.

P0395. Overexpression of exon 1B in mutant *Ptch1* transcripts in tumors of heterozygous *Ptch1*$^{neo67/+}$ mice

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Disregulation of the Sonic hedgehog (SHH) signaling pathway leads to formation of various tumors and is often caused by mutations of the tumor suppressor and SHH receptor PATCHED1 (PTCH1). The resulting tumors overexpress several downstream target genes of the pathway including *GLI1* and *PTCH1* itself. Heterozygous *Ptch1*$^{neo67/+}$ mice develop rhabdomyosarcoma (RMSs) that highly express *Ptch1*. The wild-type-*Ptch1* (*Ptch1*^{Wt})-allele is retained in these tumors, but overexpressed *Ptch1* transcripts are derived from the mutant allele. In normal muscle, *Ptch1* transcripts contain one of three alternative exon 1 sequences, which are expressed at similar levels. Here we show that the alternative *Ptch1* exon 1B, which is known to block Shh signaling, is overabundant in mutant *Ptch1*$^{\Delta 67}$-transcripts in RMS. We also show that the *Ptch1*^{Wt} transcript number in RMSs is reduced in comparison to normal skeletal muscle of heterozygous *Ptch1*$^{neo67/+}$ mice. Altogether our data suggest that overexpression of exon 1B in mutant *Ptch1*$^{\Delta 67}$-transcripts results in a mutant protein that is no longer able to inhibit the Shh signaling cascade, finally resulting in runaway activation of the pathway and tumor formation.

P0396. Somatic PTPN11 mutations in childhood Acute Leukemia

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SHP-2 is a cytoplasmic protein tyrosine phosphatase functioning as signal transducer downstream to growth factor and cytokine receptors. SHP-2 is required during development, and we demonstrated that germ-line missense mutations in PTPN11, the gene encoding SHP-2, cause Noonan syndrome (NS). Hematologic malignancies have been reported in children with NS. This association, combined with the key-role of SHP-2 in hematopoiesis, led us to consider PTPN11 involvement in leukemogenesis, and we recently documented that somatic PTPN11 defects represent a major event in juvenile myelomonocytic leukemia (JMML).
Here we extended our study on SHP-2 role in leukemogenesis by investigating the prevalence, spectrum and distribution of PTPN11 mutations in two large cohorts of children and adolescents with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). We report that PTPN11 lesions are recurrent in B-cell precursor ALL (7% of cases) and acute monocytic leukemia (25% of cases), providing evidence for a wider role of PTPN11 lesions in leukemogenesis. In the former, mutations prevalently occur in TEL-AML1-negative cases with CD19+/CD10+/cyIgM- immunophenotype. Leukemia-associated PTPN11 mutations are missense, and are predicted to result in SHP-2 gain-of-function. While PTPN11 defects identified in JMML, AML and ALL are similar to those observed in NS, the distribution of mutations differs substantially and the molecular lesions are mutually exclusive.
Our findings provide first genetic evidence of a mutated protein tyrosine phosphatase acting as oncoprotein in both lymphoid and myeloid malignancies, but also suggest a lineage- and differentiation stage-related contribution of these lesions to clonal expansion.

P0397. Establishment and long term follow-up of a sensitive interphase FISH-assay for the early detection of Fanconi Anemia-specific MDS- and AML-associated chromosomal imbalances on uncultivated bone marrow and peripheral blood cells

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Bone marrow (BM) failure, MDS and AML in Fanconi Anemia (FA) patients are strongly associated with the occurrence of FA-specific clonal aberrations in BM cells (Tönnies et al., Blood 2003). Our data revealed, that gains of 3q and following polyclonal losses of chromosome 7 represent an adverse risk factor for the progression into MDS/AML. Here we present our long-term interphase FISH study for the highly sensitive routine detection of clonal aberrations in FA cells. We screened BM and PB direct preparations (PBD) of non cycling cells, BM smears, and cultured BM cells for the presence of 3q and chromosome 7 imbalances by interphase FISH. Our long-term series included 91 FA patients with 490 single FISH analyses (500-1000 interphase cells/analysis). Healthy blood and BM donors served as controls. The comparison of BM versus PBD data showed a strong correlation by means of non-aberrant and aberrant cells. Furthermore our findings clearly demonstrate that evolving polyclonal aberrations found in BM cells are sensitive detectable in PBD of FA patients. In two FA patients, subtle 3q aberrations have been detected primarily in PBD without former bone marrow analysis. Based on this interphase FISH assay a detailed risk profile for each FA patient serves as a decision support towards bone marrow transplantation.

P0398. Analysis of *NF1* gene in sporadic colon cancer

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Tumorigenesis of colon cancer is a multistep process of mutation accumulation in a number of oncogenes and tumor suppressor genes. *NF1* protein product, neurofibromin, acts as a tumor suppressor by turning the active form of Ras into an inactive one. This molecular switch has an important role in the control of cell cycle and differentiation and the changes in the Ras activity are involved in number cancers. Several different isoforms are formed by alternative splicing of *NF1* mRNA. Isoform type I was the first isolated isoform that lacks any insertions. Isoform type II contains an additional 63 bp insertion (exon 23a). These two isoforms are expressed at varying ratios in different cell types Several studies have shown that alterations in the type I vs. type II mRNA ratio can be associated with the development of certain malignancies.
In this study we investigated the loss of heterozygosity (LOH) of the *NF1* gene in 100 sporadic colon cancers. Quantitative RT-PCR was used to determine the *NF1* mRNA expression in tumor and corresponding normal mucous tissue. Relative ratio of *NF1* types I and II expression was examined as well. The results were correlated with the patients' clinicopathological features.
LOH was observed in 20.7 % of informative samples. Expression of *NF1* mRNA was higher in well differentiated tumors and tumors classified as Dukes'A. *NF1* isoform type II was dominantly expressed in normal mucous tissue, while the isoform type I was dominantly expressed in tumor tissue.

P0399. Amplification of the RUNX1 Gene in Childhood Acute Lymphoblastic Leukemia.

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The bone marrow of seventeen pediatric patients with precursor-B cell acute lymphoblastic leukemia (ALL) was analyzed by conventional GTG bands, the FISH method using ETV6-RUNX1, 21 chromosome painting, 21q22.13-21q22.2 unique sequence probes and CGH. Conventional cytogenetic showed seven patients having normal karyotypes, while in 9, chromosomal markers or chromosome 21 tandem triplications or quadruplications were found, in some cases, associated with other abnormalities. In two patients the karyotype was not obtained. The FISH study using ETV6 and RUNX1 probes showed amplifications of the RUNX1 gene, ranging from 3 to 8 copies. The 21 chromosome painting revealed metaphases with one normal chromosome 21 and one larger size derivative 21. DNA probe for the 21q22.13 - 21q22.2 region and CGH also revealed RUNX1 amplifications. The multiple RUNX1 copies were the product of tandem duplications in chromosome 21. Analysis of major clinical and biological features revealed that all patients had B-cell precursor ALL, the median age was 8 years (range 2-15) and the peripheral white blood cells (WBCs) counts at diagnosis were low. With respect to the outcome, 3/17 patients died and three more relapsed. As far as we know 40 patients have been reported as having multiple copies of RUNX1; they share several features suggesting that they represent a specific subtype of B-cell precursor ALL.

P0400. Genetic alterations in stromal cells of malignant and borderline epithelial ovarian tumors

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There is growing evidence that tumor stroma is an active and integral part of a neoplasm. Using six microsatellite markers from 3p21.3, a region harboring potentially important tumor suppressors, allelic imbalance was found in 60-87% of the informative tumor cells microdissected from histologic sections of 58 patients with epithelial ovarian cancer. Adjacent stromal cells showed allelic imbalance at a frequency almost as high as the tumor cells (52-80%). Furthermore, laser capture microdissected epithelial and stromal compartments of borderline tumors (10) showed also a high rate of allelic imbalance both in the epithelial and stromal cells.
Another example of the importance of stroma is hyaluronan that accumulates in the peritumoral matrix of ovarian carcinomas, and acts as an independent, unfavorable prognostic factor for tumor spreading and patient survival. Allelic imbalance was not correlated with the accumulation of hyaluronan, nor clinicopathological parameters. The results suggest that factors other than inactivation of the *HYAL1-3* genes, coding for hyaluronan degrading enzymes, on 3p21.3 are responsible for hyaluronan accumulation in epithelial ovarian tumors.
More important, the results indicate that the stromal cells of the epithelial ovarian cancers not only respond to the signals from malignant epithelium, but have themselves undergone genetic alterations in regions partly identical to those in the malignant epithelial cells, and may actively contribute to the development of the tumor from its early stages to the late determinants of patient mortality.

P0401. The RING finger protein OSTL Interacts with HAX1 and SIVA: Possible Role in B Cell Signaling and Apoptosis

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The translocation t(6;12)(q23;13) results in the fusion of ETV6 with STL in a childhood B-cell acute lymphoblastic leukemia (ALL) cell line (SUP-B2). OSTL shares the first exon with STL but is transcribed in the opposite direction (OSTL = Opposite STL). Since the ETV6/STL fusion gene encodes only a very small protein, which lacks any known functional domains, we hypothesize that the main leukemogenic effect of this translocation is the deregulation of OSTL. The human STL/OSTL genomic locus spans more than 450 kbp on 6q23. OSTL encodes for a protein of 307 amino acids containing a RING finger related motif, a modified B-box domain and a RING finger motif. The OSTL protein is highly conserved: the human and mouse proteins are 99% homologous and between the human and puffer fish protein there is still 83% homology.
A yeast two hybrid screen identified HAX1 and SIVA as OSTL interacting proteins. HAX1 also co-immunoprecipitated with OSTL in an in vitro pulldown assay. HAX1 (HS1 binding protein X1) was originally identified as a binding partner of the hematopoietic cell-specific Lyn substrate1 (HCLS1, HS1)which is involved in B-cell

receptor signaling. HAX1 has also been shown to protect cells from BAX induced apoptosis. SIVA binds to the CD27 (TNFRSF7) receptor and has pro-apoptotic properties. CD27 signalling is important in B cell survival and differentiation.
These results suggest that OSTL plays a major role in the regulation of apoptosis in hematopoietic cells and supports our hypothesis that deregulated OSTL expression can lead to hematologic malignancies.

P0402. Investigation of k-ras codon 12 and p53 gene mutations in tumor tissues and surgical margins of NSCLC

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Lung cancer is the leading cause of cancer deaths in the world with over million cases diagnosed every year. Some oncogenes including K-ras and several tumor suppressor genes including p53 play an important role in the developing of lung cancer. K-ras codon 12 mutation which is the most common mutation ranges from 15-30% in lung cancer. Furthermore, p53 gene have a very important role in biological properties of the tumor cell and it is mutated in about 50% of non small cell lung cancer (NSCLC). Moreover, residual tumor cells remain in the surgical margins diagnosed as tumor-free by histopathological techniques and they can play a role in forming the local recurrence. Molecular methods may be exploited that are sensitive enough to detect small numbers of tumor cells.
In the present study, we examined p53 gene mutations and K-ras codon 12 mutations on tumor samples and surgical margins of 34 NSCLC patients by heterodublex analysis (HDA) and PCR-restricted fragment length polymorphism (RFLP) methods, respectively. P53 mutation was detected in primary tumors of 3 subjects (8,82%) by HDA. Mutations were clustured in exon 5. Moreover K-ras codon 12 mutation was detected in both primary tumor tissues and surgical margins of two out of 34 patients (5,88%) by PCR-RFLP method.Our mutation rate was determined as a very low range than literature. We think that different mechanisms related with other oncogenes or tumor supressor genes and individuals genetic differencies might be play a role in forming cancer in our study group.

P0403. Integrated analysis of SKY-, CGH- and gene expression data identifies a gene within the IL6-pathway that is lost in the IL6-independent Multiple Myeloma cell line MM.1S.

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Introduction Multiple Myeloma (MM) is a B-cell neoplasm characterized by the accumulation of clonal malignant plasma cells in the bone marrow. Although the pathogenesis of the disease still remains unclear, it is well established that interleukin-6 (IL6) plays an essential role in the malignant progression of MM. Since the existence of IL6-independent MM cells in end-stage patients may correlate with a poor response to anti-IL6 treatment, the understanding of the mechanisms that lead to IL6-independency are of crucial importance for the development of an effective MM therapy.
Goal and experimental approach To explore the mechanisms which may lead to IL6-independency, we have compared genomic (i.e. SKY- and CGH-) data from one IL6-independent MM cell line (MM.1S) and two IL6-dependent cell lines (INA-6 and ANBL-6) with proprietary as well as public [Croonquist et al., Blood. 102 (2003) 2581] gene expression data (Affymetrix) from these cells.
Results Firstly, the data demonstrate significant correlation between gains / losses of genomic material and the gene expression level in the respective areas. Secondly, we have identified a genomic region, which is present in both IL6-dependent cell lines, but absent in the IL6-independent cell line. This observation lead us to a detailed analysis of the expression pattern of the genes located in that region. Interestingly, we found one gene, that i) is known to be involved in the IL6-pathway and ii) is only expressed in the IL6-dependent cell lines, whereas no expression of this gene could be detected in the IL6-independent cell line MM.1S.

P0404. Genome-wide analysis of acute myeloid leukemia with normal karyotype by using microarray-based comparative genomic hybridization

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Acute myeloid leukemia (AML) is a heterogeneous disease for which cytogenetics is one of the most important prognostic factors, since cytogenetic abnormalities provides valuable information of diagnostic and prognostic relevance. An abnormal karyotype is found in 54-78% of AML at presentation. Although molecular genetics detects more aberrant cases than metaphase cytogenetics, the percentage of cases with apparent normal karyotype at presentation is high.
We used a molecular cytogenetic technique called microarray-based comparative genomic hybridization (Array CGH) to search for submicroscopical gains or losses in 14 AML cases with normal karyotype after G-banding. Six patients had an AML-M1 (myeloblastic leukemia without maturation) and eight had an AML-M2 (myeloblastic leukemia with maturation). Using a 1Mb whole genome chip, chromosomal abnormalities were detected in 5 patients with AML-M1 and in 1 patient with AML-M2. None of the aberrations was greater than the size of 1 BAC/PAC. There were 11 gains and 1 loss of chromosomal material. Gains were on Y chromosome, 8q, 9q, and17q. The only deletion was on 15q. All abnormalities we found were unique. The loss on 15q could not been validated by using FISH technique. FISH was not the suitable technique to validate such small gains, since with this technique it is no possible to quantify the intensity of the signals.In conclusion we suggest to reexamine AML cases with apparent normal karyotype for submicroscopic alterations by using array CGH. The recurrence and clinical relevance of the abnormalities detected in our cases need to be proved in a larger collective.

P0405. Chromosomal aberrations in follicular Non-Hodgkin-Lymphomas (NHL) of Japanese patients detected by PCR and CGH analysis

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The incidence of follicular lymphoma (FL) as well as the frequency of the translocation t(14;18)(q32;q21) in FL-patients is lower in Asia compared to Western countries. The question was raised if this is due to a different molecular pathogenesis. Therefore, we have done the first study in 23 Japanese FL-patients using both comparative genomic hybridization (CGH) and PCR analysis of t(14;18) with primers for the major breakpoint (MBR), minor and intermediate cluster region (mcr and icr) on 33 biopsy samples.
In 18/23 patients, CGH analyses detected genomic imbalances. Gains affected most frequently chromosomes 18p (6/23), X (5/23), 5 (4/23), 12 (4/23), 2 (3/23), and 16 (3/23). The only recurrent loss affected chromosome 6q (2/23). PCR analysis for t(14;18) revealed in 13/23 patients breakpoints in the MBR (n=10), 3'MBR (n=1), mcr (n=1), and icr (n=1). Correlating CGH with PCR data, recurrent aberrations were more frequent in primary samples from patients with t(14;18).
In summary, we showed that there is no difference in the overall frequency of aberrations compared to previous studies on westerners, but the frequency of t(14;18) was lower. Additional studies are required to assess the reason for geographic variation in the incidence of FL.

P0406. Association of common RASSF1A polymorphisms with breast cancer

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The RASSF1A tumor suppressor gene, located at 3p21.3, is found to be polymorphic in patients with breast cancer, however the presence and incidence of polymorphic RASSF1A has not been evaluated. We studied the association between 2 common single nucleotide polymorphisms in the RASSF1A gene (at codons 28 and 133) and risk of breast cancer in a group of 220 cases and 107 controls from Sachsen-Anhalt, Germany. The histological examination of the tumor probes showed following findings: 140 carcinomas and 80 other alterations (fibroadenomas, mastopathy, fibrosis or hypertrophy). To genotype these polymorphic variants we used SSCP and sequencing. The distribution of the CGT to CGA polymorphism (silent) at codon 28 showed no significant difference between the two groups (A allel carriers: controls 1,9 % and patients 4,3 %). However the GCT to TCT polymorphism at codon 133 (A133S), which alters a potential phosphorylation site of the ATM kinase, exhibited a different genotype distribution. The patients with carcinoma had more mutated T allel (29/140, 20,7 %) than the control group (17/107, 15,9 %). The highest frequency of the T allel was found in fibroadenomas (4/12, 33,3 %). In contrast, the lowest rate of this allel was detected in cases with mastopathy (2/41, 4,9 %, p<0,03). Our data demonstrates that polymorphic RASSF1A at codon 133 occurs in an increased number of breast tumors, especially in fibroadenomas. Thus, alteration of the RASSF1A genotype may play a role in the pathogenesis of breast cancer. This study was supported by BMBF.

P0407. Multicolor banding fluorescence in situ hybridization (mBAND) technique in the evaluation of 5q deletions.

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Exact frequency of del(5)(q31) in bone marrow cells of patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) can be established only by FISH with locus specific probes for 5q31 region. Deletion can be present as sole aberration and/or as one of complex karyotype rearrangements. Del(5)(q31) is thought to occur in myeloid precursor cell as a primary chromosomal defect, which is in close relationship with the origin of the disease. The exact breakpoints are sometimes difficult to determine by G-banding. **The aim** of this study was to reveal the breakpoints of del(5)(q) by mBAND and to establish the most frequent type of deletion with possible correlation between cytogenetic findings and clinical parameters. We present 22 patients with MDS or AML all studied with locus specific probe 5q31(VYSIS™) and mBAND with the XCyte5 probe kit (MetaSystems™). Suspected rearrangements of 5q were examined by multicolor FISH (mFISH) (three cases). mBAND revealed that the most common type of deletion was del(5)(q13.3q33.3) (six cases). In all other patients the breakpoints differed from case to case. Unusual interstitial deletion with 5q31 region conserved on deleted chromosome 5 was found in one female with MDS-del(5)(q14q23.3). It was proved by mBAND and mFISH that 5q deletions appear in variety of forms with certain clinical correlations found between different types of aberrations. mBAND is highly recommended to be utilized in all cases where the extent of the deletion cannot be proved by classical banding techniques. Supported by grants GACR 301/01/0200 and GACR 301/04/0407.

P0408. Detection of chromosome 13 abnormalities and 14q32 translocations in multiple myeloma using simultaneous immunofluorescent labelling of malignat plasma cells and fluorescent in situ hybridization

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Chromosomal aberrations such as 13q14 deletions or translocations involving 14q32 are described to be common cytogenetic findings in multiple myeloma (MM).
Especially, deletions of 13q14 has been associated with an adverse outcome and it has been proposed as one of the most important prognostic factors for MM patients.
Because metaphase cytogenetic studies in MM are hampered by a low proliferative activity of myeloma cells, interphase fluorescent in situ hybridization (FISH) using specific DNA probes is the technique most widely used for the determination of genomic aberrations in this disease.
In the present study we have performed FISH experiments with probes directed to the 13q14 and 14q32 chromosomal regions in 30 patients with MM.
For identification of malignant plasma cells in bone marrow samples, we have used cytoplasmic immunoglobulin (cIg) labelling methodology (Ahmann et al. 1998). This method allowed us to identify simultaneously monotypic plasma cells by monoclonal antibody fluorescence (AMCA anti-kappa or anti-lambda chain) and detect chromosomal abnormalities by FISH (cIg-FISH).
FISH studies revealed that monoallelic deletions of 13q14 or monosomy 13 were present in 15 of 30 (50 %) patients, 14q32 abnormalities were observed in 9 of 30 (33 %) patients with MM.
Our results confirmed that by combining immunofluorescent labelling of myeloma cells and FISH, 13q14 deletion can be proved also in patients with apparently normal karyotype in unselected bone marrow sample.
We conclude that cIg-FISH procedure represents simple and reliable methods that can increase the incidence of chromosomal abnormalities required for prognostic evaluations of patients with MM.

P0409. Mutation analysis of MUTYH by DHPLC in autosomal recessive FAP families

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Familial adenomatous polyposis (FAP) is a premalignant disease characterized by adenomatous polyps of the colon and rectum. The number of polyps can range between a myriad of polyps in extreme cases to 10 or less. One or more polyps progress through dysplasia to malignancy, leading to cancer at any age from late childhood through the seventh decade.
The autosomal dominant FAP is associated with mutations in the APC (adenomatous polyposis coli) tumor-supressor gene. It has recently been demonstrated that autosomal recessive FAP is caused by mutations in the base excision repair DNA glycosylase MUTYH encoding a component of the mismatch repair system of oxidatively damaged DNA.
We established mutation analysis for MUTYH using denaturing high performance liquid chromatography (DHPLC). Deviations were confirmed by direct DNA sequencing. In addition, sequencing has been performed for the two exons with known homozygous mutations.
We screened for germline MUTYH mutations in 40 APC mutation-negative patients with multiple (3 to >100) colorectal adenomas and mostly without positive family history. Here, we report 2 patients with compound heterozygous MUTYH germline mutations and 5 patients with monoallelic heterozygote missense or nonsense mutations. These include two new MUTYH variants: a missense mutation (E476K) and one mutation resulting in deletion of one amino acid del476E.

P0410. Mutation screening of APC gene in Czech FAP patients

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Germ-line mutations in the adenomatous polyposis coli (APC) gene are responsible for familial adenomatous polyposis (FAP). FAP is a dominantly inherited colorectal tumour predisposition, which shows substantial phenotypic variability. Patients with classical polyposis develop more than 100 colorectal adenomas, whereas those with multiple adenomas have fewer than 100 adenomas. The later

patients are classified as attenuated familial polyposis (AFAP) or as “multiple” adenomas patients. Mutation screening involves the entire APC coding region using PCR, PTT, DGGE methods and sequence analysis. This study examined 82 unrelated individuals with colorectal polyposis. Germ-line mutations were detected in 57 (69,5%) probands. Of these, 20 (35%) were detected in exons 1-14 and 37 (65%) in exon 15. Altogether, we report 30 previously reported mutations and 27 novel mutations, which are unique for Czech population. The most commonly reported mutations at codon 1309 were found in 5 (9%) and at codon 1061 in 3 (5%) of the patients examined. Additionally, we detected a relatively higher proportion of mutations at codons 935 (7%), 541 (5%) and 213 (5%). Routine mutation detection techniques fail to detect APC germ-line mutation in approximately 30% of patients with classical polyposis and 90% of those with AFAP/multiple adenomas. The failure to detect germ-line mutations might be caused by large submicroscopic deletions or by mutations in other candidate genes. In our set of patients the biallelic germ-line mutation in the MYH gene was detected as we previously reported.

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P0411. Clinical and molecular characteristics of hereditary breast or/and ovarian cancer in Russian population

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Frequently hereditary gynecologic cancers are present as site-specific breast cancer, breast/ovarian cancer syndrome and site-specific ovarian cancer and are confirmed by testing of cancer-prone genes. Germline mutations of BRCA genes are independent molecular markers of these disorders.

Spectrum, frequency and penetration of mutations and sequence variations of BRCA-associated breast or/and ovarian cancer were analyzed. We selected 114 individuals (98 families), from it 34 site-specific breast cancer patients (s-s BC), 21 breast/ovarian cancer patients (BOC) and 22 site-specific ovarian cancer patients (s-s OC) and 37 healthy first/second degree relatives. The entire coding regions of BRCA 1 and BRCA 2 genes were screened by conformation sensitive gel electrophoresis (CSGE). All structural variants of amplified DNA fragments were sequenced on both strands.Among probands 7 BRCA 1 mutations and set of 8 SNPs that are inherited as a haplotypes of BRCA 1 gene were found, 6 mutations and 6 SNPs of BRCA 2 gene. S-s BC in 47.0% (16/34) was associated with BRCA 1 mutations and SNP of BRCA 1/2; in 35.3% (12/34) of case - with mutations and SNP of BRCA 2. 12 BOC patients (57.1%) were carriers of pathologic BRCA 1/2 genotype. BRCA 1 germline mutations have been identified in 17 (77.2%) affected s-s OC patients. High frequency of mutations 5382 insC BRCA 1 (76.4% of all mutations) was shown. About 52% of s-s BC / BOC patients were positive by the env MMTV gene-like sequences. Changes in BRCA 1/2 genes have been found in 11 (29.8%) healthy relatives.

P0412. Tissue microarray analysis of epidermal growth factor receptor gene in Bulgarian patients with colorectal carcinoma

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Tissue microarray analyses are a quite new and promising technique which allows investigation of different quantitative genetic changes in numerous samples. With fluorescent in situ hybridization (FISH) on tissue microarray can be analysed such changes of specific genes involved in cancer development pointing out to a possible correlation of amplification of these genes with tumour progression and patient prognosis.

We have analysed 243 tumour samples with different stage, grade and localization (colon, sigma and rectum) for epidermal growth factor receptor (EGFR) gene amplification. All the patients were with sporadic form of the disease.

The aim of the study was to find out if the EGFR gene is amplified in order to assess its connection with colorectal cancer development. FISH was performed using dual colour LSI EGFR spectrum orange (for detection of EGFR gene signals) / CEP 7 spectrum green (for centromers) probe. The analysis showed neither an amplification of EGFR gene in tumour cells nor gain (number of gene copies less than three times of centromer signals).

From the present study, we can conclude that the tumour characteristics, its development and phenotype in the case of colon cancer are not in association with quantitative changes of EGFR gene. In this case EGFR gene can’t be taken as a prognostic marker of the colon cancer development.

P0413. HNPCC: genomic deletions in MLH1 and MSH2 in 10% of the patients

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Hereditary nonpolyposis colorectal cancer (HNPCC) is the most frequent autosomal dominant predisposition for early-onset colorectal cancer caused by germline mutations in DNA mismatch repair genes. We studied a cohort of 420 colorectal cancer families selected on the basis of the Amsterdam and the Bethesda criteria. Within this cohort, we found 64 patients with MSI-H tumors and loss of expression for either MLH1, MSH2 or MSH6 but without detectable mutation in one of these genes. To determine the relative copy number of each MLH1 and MSH2 exon we performed multiplex ligation-dependent probe amplification (MLPA) method from MRC Holland. We found 8 different genomic deletions in 9 unrelated families, 7 in MSH2 and 1 in MLH1. This equals 10% of the mutation-negative cohort. Evidently, genomic deletions in MSH2 and to a lesser extent in MLH1 can cause HNPCC in German colorectal cancer families. Thus, screening for genomic rearrangements in MSH2 and MLH1 should be a further step in the molecular diagnosis of HNPCC. However, beside point mutations and deletions in MLH1, MSH2 or MSH6, other mutation mechanisms must occur, as no mutations were found for 52 patients with pathologic results in immunohistochemistry and microsatellite analysis.

P0414. Unexpected chromosomal rearrangement t(6;14) identified in a case with myelodysplastic/myeloproliferative disorder at first presentation

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Recently proposed WHO category myelodysplastic/myeloproliferative (MDS/MPD) diseases exhibit both dysplastic and proliferative features at the time of first presentation.

We report the case of a 59-year old man who was admitted to the Coltea Clinical Hospital - Department of Hematology and in whom hematological and cytogenetic investigations discovered splenomegaly, abnormal blood and marrow findings and structural chromosomal abnormalities.

Peripheral blood cell count revealed thrombocytopenia, anemia and leukocytosis with circulating myeloid blasts, basophilia and eosinophilia. The bone marrow biopsy performed at diagnosis described an aspect consistent with MDS/MPD.

Cytogenetic investigation was performed on GTG-banded chromosomes obtained after short-term cultures, from bone marrow sample, using classical methods. Several structural rearrangements were identified: translocation t(6;14)(q21;q32), del(11)(p?) and also a genomic instability with the presence of chromosome breaks and double minutes.

Although recently recognized, MDS/MPD category represents a challenge due to difficulties in diagnosis and poor prognosis. The cytogenetic investigation of these cases may bring useful data for a better understanding of pathogenetic mechanisms involved.

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P0415. Mitochondrial heteroplasmy in hematologic malignancies

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MtDNA is only transmitted through the maternal line and is not subject to the mechanism of recombination: mutation is thus the only possibility of genetic diversity. It has been suggested that MtDNA is involved in carcinogenesis, because of its high rate of mutations and limited repair mechanisms. The most frequent genetic defects of the mitochondrial genome are due to point mutations rather than deletions, and pathogenetic mutations are usually heteroplasmic [Lightowlers RN et al, Trends Genet, 1997;13:450]. It has also been observed that the substitution rate for the control region is not significantly different from that for the coding region [Cavelier et al, Hum Genet, 2000;107:45]

The aim of this study was to gain knowledge of mtDNA heteroplasmy conditions in families and the possibility of etiological correlation between a particular mitochondrial genetic profile and cancers of various kinds. The mtDNA control region was studied by sequence analysis in capillary electrophoresis of blood samples from 25 mother-child pairs, from 50 patients with hematological neoplastic pathologies and subjected to bone marrow transplant, and from their healthy sibling donors.

This study revealed a higher percentage of heteroplasmy in blood samples from individuals affected by hematological diseases and in their donors, with respect to blood samples from normal cells of mother-child pairs. Cases of heteroplasmy identified in sibling pairs involved length and sequence heteroplasmy conditions.

P0416. Hypermethylation of the CpG islands in the promoter region flanking GSTP1 gene is a potential plasma DNA biomarker for detecting prostate carcinoma

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Purpose: to investigate whether our newly developed methylation-sensitive real-time quantitative PCR protocol could be used to detect cancer genomes in prostate cancer patients' circulation. Study design: Plasma or serum DNA samples were extracted from thirty-six prostate cancer patients and 27 benign prostate hyperplasia (BPH) cases. After extensive methylation-sensitive restriction enzyme, HpaII, digestion, the DNA samples were subjected to the real-time quantitative PCR amplification. The dissociation curves analysis was applied to know if hypermethylation occurred in the promoter region flanking the GSTP1 gene among these patients. Results: 11 of 36 prostate cancer patients showed positive peak pattern, indicating methylation changes occurred. However, 25 of the 27 BPH cases showed no peak, suggesting no methylation changes happened in the CpG islands these cases. The sensitivity and specificity of this protocol performed on plasma or serum samples were 31% and 93%, respectively. Conclusions: We have successfully analyzed prostate cancer genome in the peripheral blood in 31% prostate cancer patients with this protocol. This method can effectively distinguish BPH from prostate neoplasm. Using plasma or serum DNA samples is an ideal noninvasive detection of prostate neoplasm or for treatment follow-up, when enough amounts of cancer genomes presented in the circulation.

P0417. Unfavourable translocation t(13;21) in a case of AML-M2

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Cytogenetic investigation performed by classical GTG-banding in 13 patients diagnosed with different types of AML, revealed normal karyotype in 15% of them. The other 85% of AML cases exhibited complex chromosomal aberrations.

Among the 13 AML cases, one young man, aged 28, exhibited an unusual translocation t(13;21), with an adverse prognosis. The patient died within 5 months from diagnosis.

The most characteristic chromosomal aberration in AML-M2 is t(8;21), but there are reported few cases with der(13)(q10;q10) (van Limbergen et al 2002, Yaspo et al 1992) as a part of complex karyotypes.

It remains still unclear whether an association between t(13;21) and adverse prognostic in AML-M2 is possible. Anyway, this finding underlines the fact that, apart of classical cytogenetic rearrangement already assigned to a type of leukemia, every patient may exhibit new rearrangements of cellular genome. These aberrations need to be characterized in order to elucidate their involvement in pathogenesis of malignant myeloid disorders.

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P0418. Genomic profiling of gangliogliomas

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Gangliogliomas (GG) are neuroepithelial tumours frequently associated with epilepsy. They are composed of dysplastic neuronal as well as neoplastic glial elements and are generally benign (WHO grade I or II). Little is known about their molecular pathogenesis. In this study, we aimed to identify chromosomal regions involved in GG tumorigenesis. Sixty-three GGs (57 WHO grade I, 6 WHO grade II) were screened for chromosomal imbalances by comparative genomic hybridization (CGH). Chromosomal aberrations were detected in 22 of 63 GGs (35%). The number of aberrations per tumour ranged from 1 to 11 with an average of 2.32±0.51 (mean±SEM). Recurrent gains were identified on chromosome 7 (6/63); 8 and X (5/63 each); 5 (4/63); 12q (3/63); 9q, 17, 19 and 20 (2/63 each). Recurrent losses were found on chromosomes 9 (5/63); 10 and 16 (2/63 each); Y (2/33 tumours from male patients). Combined gains on 7, 8 and 5 or 12q were detected in 4 cases. To achieve a higher resolution in the detection of genomic imbalances, 7 tumours were analyzed by matrix-CGH. A whole genome array of 6000 BACs/PACs with a resolution of at least 1Mb was used. In all cases, the results obtained by chromosomal-CGH were confirmed. In 3 of 7 GGs, additional aberrations could be identified by matrix-CGH. In conclusion, our study provides the first comprehensive overview of DNA copy number changes in gangliogliomas. The ongoing investigations show that matrix-CGH improves genomic resolution and can be informative even for aberrations in cell subpopulations.

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P0419. Characterisation of cytogenetic subgroups in myelodysplastic syndromes with complex chromosome rearrangements

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The presence of a complex rearranged karyotype (CCA) in bone marrow cells of patients with myelodysplastic syndromes (MDS) or acute myeloid leukaemia (AML) has a negative impact on the individual risk stratification following the international prognostic scorings system (IPSS), regardless of the nature of the aberrations. We performed a comprehensive molecular cytogenetic analysis including SKY and FISH on 16 patients with primary MDS and four additional patients with primary AML. All patients had CCA by conventional cytogenetic analysis at first diagnosis of the haematological disease. In addition to SKY we have performed FISH analysis with probes from chromosome 5q in all patients and from the MLL- and AML1-gene in selected cases. WCP-probes for chromosomes 5 and 21 were used to verify the SKY results. All patients with deletions of chromosome 5 showed loss of both critical regions for MDS and AML in 5q31and q33. 10 patients had interstitial deletions of chromosome 5q of various extend and 10 patients had an unbalanced translocation with breakpoints in 5q resulting in loss of 5q material. The translocation breakpoints were shown to be heterogeneous using BAC-probes mapping to each band on 5q. The

most common imbalances of genetic material were: -5q (20/20), -7/7q (14/20),
-18/18p (8/20) and -16 (6/20), complete trisomy 8 (5/20), +21q (5/20), +11q (3/20) and +22q (3/20). Interestingly in patients with gains of chromosome 11 or 21 amplifications of the MLL or AML1 genes respectively were excluded. A cluster analysis of the SKY results identified two main cytogenetic subgroups.

P0420. Genetic variability between clonal lines of tumor hepatocytes with various differentiation under different conditions of cultivation revealed by RAPD-PCR

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It is of a special interest to find out to what degree the capability of tumor cells for differentiation and loss of malignancy depends on genetic variability taking place at cell malignant transformation and tumor progression. Unlike the earlier used methods of studies of genome instability, the RAPD-PCR method allows detecting the most variable DNA fragments and revealing genes or their fragments in ampliphicated DNA fragments.
Using the RAPD-PCR method, genetic variability in clonal lines of mouse hepatoma MH-22a was studied at their proliferation in subcutaneous connective tissue (SCT) and anterior chamber of the eye (ACE). The comparative analysis of the genetic structure of the clonal line populations in vitro and in vivo has revealed that the amount of clones with the high, intermediate, and low variability is approximately the same in both cases. It was also shown that indexes of genetic variability (GVI) in various clonal lines in vitro correlated with their vital ability: the clones yielding clone lines had the lowest GVI. The same GVI value was found in the clonal lines proliferated in the ACE regardless of their capability for differentiation. Intraclonal analysis has shown that the highest values of changes revealed on fingerprints of the amplification of DNA products do not prevent from differentiation of tumor hepatocytes in the ACE.
These data allow concluding that tumor cells can preserve ability for differentiation in spite of significant changes in their genome.

P0421. Amplification of the BCR/ABL1 fusion gene after Imatinib - one way of drug resistance in CML therapy

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The recently developed tyrosinkinase inhibitor STI571 (Gleveec, Imatinib) led to a significant improvement of the therapy of CML, AML and ALL. However, drug resistance can be observed in 4% of CML patients in chronic phase during therapy. Mutations of the tyrosine kinase domain has been detected as one way of this drug resistance. We describe a second way caused by an amplification of the BCR/ABL1-fusion protein. We report on a 54 years old male patient with CML. Since four years he is staying in chronic phase. The therapy was changed from conventional chemotherapy to Imatinib three years after diagnosis. Conventional cytogenetic analysis (CCA) and fluorescence in situ hybridisation (FISH) with the D-FISH probe [Q BIOgene] were performed on unstimulated bone marrow cells. At the time of diagnosis and one year later CCA and FISH revealed 46,XY,t(9;22) and BCR/ABL1-rearrangement in all cells. 15 months later two additional clones with add(6)(p) and add(19)(p) could be detected. Therefore Imatinib therapy was initiated. Four years after diagnosis CCA showed altogether five different clones and FISH detected four different clones with up to five fusion signals, one on the der(9) and up to four on the derivative chromosomes 22. One to two of the derivative chromosomes 22 could be identified as isochromosome 22 [i(22)(q11)t(9;22)(q34;q11)]. Although the patient was treated with Imatinib an increase of the aberrant clones with i(22)-chromosomes was detected. In contrast to the typical Philadelphia-chromosome the i(22)-chromosomes showed an increased fusion signal intensity, suggesting an amplification of the BCR/ABL1-fusion product.

P0422. Assessment of gene expression profilings 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 contribute to tumor and metastases development. It has been stressed that different factors such as VEGF, which play a key role in neoplastic angiogenesis are also very important for tumor growth. The role of DCC and BRCA genes is still unclear but it is highly possible that these genes, in some extends, could be involved in endometrial cancer etiopathogenesis. Complexity of molecular mechanisms leading to endometrial cancer development implies that only experiments utilizing array technique can collect enough data to understand all relationships between different molecular pathways during tumor development and to identify new clinically usable markers of neoplasm.
Using MacroArray technique we analyzed expression of more than thousand different genes involved in oncogenesis such as oncogenes, tumor suppressor genes and others encoding cell cycle regulators, growth factors, growth factor receptors and proteins crucial for apoptosis and extracellular matrix maintaining. Comparison of obtaind data with tumor grade allowed us to give shape to preliminary gene expression profilings reflecting tumor progression. Further analysis based on gene expression profiling and performed on protein level can reveal new markers of endometrial cancer useful in routine diagnostic procedures.

P0423. *PAX5* and *TP53* analysis in superficial bladder carcinoma tissue. Correlation with pathological findings and clinical outcome.

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The expression pattern of *PAX5* in the tissue of superficial bladder transitional cell carcinoma (TCC), its prognostic value and its correlation with TP53 immunohistochemistry and *TP53* mutation analysis were evaluated. 61 patients with histologically confirmed superficial bladder TCC were involved to the study. Expression level of *PAX5* was evaluated by means of the RT-PCR semiquantitatively. *TP53* mutations were identified by the SSCP and confirmed by direct sequencing. The TP53 imunohistochemistry was performed with the DO1 antibody and semiquatitatively evaluated using the HSCORE (HS) method. 8 men with benign prostatic hyperplasia served as control group for *PAX5* expression. Detectable *PAX5* expression was found by 50 patients with TCC but in no patient from the control group. Its quantity however correlated neither with the stage nor with the grade of the tumour. *TP53* mutations were confirmed by 7 patients. On the contrary, positive immunohistochemical staining of TP53 was detected in most patients. Using cutoff value of HS 200, 57% of patients showed *TP53* overexpression. Quantity of TP53 immunohistochemical positivity did not correlate with the quantity of *PAX5* expression. Using the cutoff values of HS 200 for TP53 and 0.2 for *PAX5*, 7 of 8 patients with future progression had TP53 and 4 had *PAX5* overexpression respectively. The *PAX5* gene expression is a frequent finding in the TCC. From prognostic point of view, high expressions of both *PAX5* and *TP53* seem to be related to a higher recurrence and progression rates. Supported by the grant IGA MZ NC/5961-3 and IGA MZ 7519-3.

P0424. Genetic analysis of the EZH2 gene in prostate cancer

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Genetic alterations of chromosome 7 have been implicated in the

prostatic tumor progression. It is supposed that overexpression of the EZH2 (enhancer of zeste homolog 2) gene, a polycomb group transcriptional repressor located on chromosome 7q35, is involved in the development of metastases. Furthermore, we had shown that there is linkage of prostate cancer to chromosome 7q31-33 particularly in 10 families with aggressive disease. We screened these 10 families encompassing 24 patients for changes within the EZH2 gene by exon sequencing in order to assess if there is a genetic predisposition for the aggressive form of prostate cancer due to a genetic variant of EZH2. No mutations were observed in the open reading frame of EZH2. Next, we conducted an association study with controls, sporadic cases and familial cases to evaluate if there is an association between the disease and the polymorphisms found in the intronic sequences of the EZH2 gene. No single SNP showed evidence of association, even taking into account the TNMG classification and the follow up of the patients. In the ongoing study we investigate if a certain haplotype of the EZH2 gene may be predisposing for the formation or progression of prostate cancer. Presently, we are extending this approach to the promoter of EZH2. In order to search for promoter variations, we are actually sequencing the 5` region of EZH2 of the 24 patients.

P0425. Comparative genomic hybridization in diffuse astrocytoma - a WHO grade correlated meta-analysis of 399 cases

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A lot of CGH data about genetic imbalances in astrocytic astrocytoma has been accumulated so far. Analysing only limited case numbers some studies revealed genetic aberrations probably associated with malignant progression. In order to perform a statistical meta-analysis of genomic aberrations in astrocytoma we summarized and analysed the CGH results of 399 cases published between 1992 and 2003. After the expansion of all aberrations to the 850-bands level the specific aberration patterns of WHO grade II, III and IV astrocytoma were calculated. Next we quantified the differences of these aberration patterns between low grade and anaplastic astrocytoma as well as between anaplastic astrocytoma and glioblastoma multiforme. For this the relative frequencies of aberrations of the lower graded malignancy were subtracted from that of the higher grade of malignancy for each GTG-band of the tumor genome.
For instance, in low grade astrocytoma gains of 7q32 and 8q24 were the most prominent aberrations. Anaplastic astrocytoma showed a tremendous increase in the aberration frequency with gains of 1q32, 7q32, 12p13, 17q24-q25, 20q and losses of 9p, 10, 13q21-q22. Glioblastoma multiforme showed a higher frequency of +7, +19p, -10 and –13, but also a shift of the dominant peak on chromosome 7 from 7q32 to 7p12 (EGFR- locus). The method presented here enables the quantification of differences of aberration patterns and to verify predicted markers of malignant progression in a larger number of cases leading to a higher degree of significance.

P0426. Cytogenetical Follow-up of Patients with Chronic Myeloid Leukemia Ph[1]+ treated with Glivec

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Glivec[R] is the first of a new generation of drugs which blocks the effect of the oncoprotein that is coded by the chimera gene bcr-abl given by the translocation t(9;22) characteristically for LMC.In our center, the treatment with Glivec[R] for LMC patients, has been initiated since the year 2002.
We folowed: clinical-hematological and cytogenetically response.We have performed a cytogenetic study in 24h non-stimulated cultures of bone marrow cells from 12 pts with CML at diagnosis and during the treatment. The GTG analysis revealed the karyotypes. We have considered: complete cytogenetical response (CCR) if there are 0%Ph[1]+ metaphases, major cytogenetically response (MCR) if there are less than 35%Ph[1]+ mitosis, minor cytogenetically response (mCR) if between 35-95% Ph[1]+ and absent cytogenetically response if 100%Ph[1]+ mitosis. All the 10 pts with chronic phase have shown classical t(9;22) in 100% of the metaphases. The 2 cases with accelerated phase have shown additional chromosomical aberrations. The pts have been given the standard doses.The cytogenetically evaluation was made at 6, 12 and 18 months. After 6 months at two pts CCR has been attained and in the rest, mCR has been noticed. After 12 months better results have been attained. If after 12 months the lack of MCR is still noticed the doses will be upgraded.
These results confirm Glivec[R] efficiency in controlling the Ph[1]+ clone. The impact of such an effect on the survival rate however requires a long term confirmation.
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P0427. Differential regulation of different 5'-end variants of the oncogene EVI1

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The EVI1 gene has been implicated as an oncogene in myeloid leukemia. Its overexpression appears to confer a particularly bad prognosis to the affected patients. Recently, EVI1 has been found to exhibit great variability at its 5'-end. While some of the alternative transcripts give rise to alternative protein products, others differ from each other only in their 5'-untranslated sequences. This suggests that EVI1 may be subject to complex regulatory mechanisms that could act on both the transcriptional and posttranscriptional levels.
In this study, we began to adress the role of this 5'-end variability for the regulation of EVI1 expression. We assessed steady state levels of the different transcript variants in a variety of human tissues, and in response to the only EVI1-inducing agent known to date, all-trans retinoic acid. Our data show that the EVI1 mRNA variants are differentially expressed under these experimental conditions. As a next step, we compared the stability of the different mRNA variants, and found that certain transcript types had significantly longer half lives than others. The highly variable length of the different 5'-untranslated regions furthermore suggests that they differ in their translation efficiency, a hypothesis currently under investigation.

P0428. Comparing methylation profile of *p16, MLH1* and *RB1* in cervical carcinoma in situ, cervical nondisplasia and cervical inflammatory diseases

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DNA methylation of CpG sites in the promoter regions of tumor suppressor genes is a frequent epigenetic event in the pathogenesis of cancers. Methylated DNA also can be a predicting factor for monitoring the onset of cancer.
We analysed 55 high-grade CIN or CIN3 (severe dysplasia and carcinoma in situ) samples and 67 cervical smears from women with inflammatory process. 37 nondisplasia tissues adjacent to carcinoma in situ, 31 preoperative blood samples and 90 blood samples of healthy donors were also evaluated. Methylation status of 3 genes (*p16, MLH1* and *RB1*) was determined using methylation-sensitive PCR.
There is no methylation determined in peripheral lymphocytes in the blood of healthy donors, which was suggested as control. Methylation percentages of the genes in CIN3 were: *p16*, 56%; *MLH1*, 46%; *RB*, 16,7%. Methylation percentages in nondisplasia adjacent tissues were statistically lower, except for *RB1*: *p16*, 34,6%; *MLH1*, 18,5%, *RB1*, 11%. In inflammatory cases, promoter methylation was detected at a statistically significant lower frequency than in the carcinoma cases, but significantly higher than in blood of healthy donors, except for *MLH:* (*p16*, 7,58% versus 0%, P=0.01; *RB1*, 6,15% versus 0%, P=0,03; *MLH1*, 3% versus 0%, P=0,18). Methylation percentages of the genes in preoperative blood were: p16, 2,7%; MLH1, 5,6%; RB, 0%.
The epigenetic alterations found in nondisplasia tissues adjacent to carcinoma in situ and in inflammatory tissues suggest their

participation in the transforming of the cells, but not necessarily in malignancy, although it can help identify groups of increased risk of cancer development.

P0429. Functional analyses of Bax inhibitor-1 (BI-1) in human prostate and breast cancer cells

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Recently, the novel anti-apoptotic gene Bax inhibitor-1 (BI-1) was shown to be overexpressed in prostate cancer and its specific down-regulation by RNA interference leads to cell death in human prostate carcinoma cells. These findings clearly indicate that the human BI-1 gene contains the potential to serve as a prostate cancer expression marker and as a potential target for developing therapeutic strategies for prostate cancer. In this report, to evaluate the pattern of BI-1 expression in different human cancers, the Cancer Profiling Array technique was applied and revealed an up-regulation of BI-1 in breast, uterus, and ovarian cancer, whereas down-regulation of BI-1 expression was determined in stomach, colon, kidney, lung and rectum cancer. Furthermore, BI-1 was shown to be expressed in six different human breast cancer cell lines. Next, to investigate the function of BI-1 *in vitro*, RNA interference (RNAi) or RNA silencing was applied for a specific down-regulation of BI-1 expression in estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cells. Suppression of BI-1 expression caused a significant increase in spontaneous apoptosis in MDA-MB-231 cells, whereas MCF-7 cells remained unaffected. After determination of BI-1 function *in vitro* in both prostate and breast cancer cells, we are currently investigating the potential of duplex siRNA oligonucleotides against BI-1 gene expression for the suppression of tumor growth *in vivo*.

P0430. TNR/11q#1 trinucleotide (GCC)n repeat alleles and predisposition to acute and chronic leukemia

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TNR/11q#1 is a polymorphic trinucleotide (GCC)n repeat located within the minimal region of 11q deletion in chronic lymphocytic leukemia (CLL). It was recently shown that certain alleles of this repeat are associated with worse prognosis in CLL patients. To investigate the role of TNR/11q#1 variants as risk-modifying factors in leukemogenesis, we conducted a case-control study on 113 acute lymphatic leukemia (ALL) patients, 82 CLL patients and 146 healthy controls of Russian origin. Comparison of allele and genotype distributions in control, ALL and CLL groups performed by Fisher's exact test with two-sided P-value showed significant decrease in the presence of GCC6 allele in ALL and CLL compared to controls. Moreover, 'rare' alleles GCC7-8 and GCC13-14 were significantly overrepresented in ALL versus control. We found that CLL risk genotypes were those with both alleles containing more than 6 GCC repeats (P=0,0212, odds ratio=1,68 (95% CI 1,121∑ 2,531)). ALL risk genotypes include three allele combination variants: 1) both alleles containing more than 6 GCC repeats (P=0,0019, odds ratio=1,756 (95% CI 1,223∑ 2,502)); 2) one of the alleles containing 7 or 8 repeats (P=0,0155, odds ratio=18,22 (95% CI 1,93∑ 136.37)), 3) one of the alleles containing more than 12 repeats (P=0,0209, Odds ratio=2,599 (95% CI 1,161∑ 5,815)).
Considering these data altogether, we resume that TNR/11q#1 is linked to a gene whose alterations create a strong predisposition to clinically severe forms of leukemia and may be lethal, thus creating a natural selection of certain alleles and leading to the disturbance of the Hardy-Weinberg equilibrium.

P0431. Glutathione S-transferase (GSTM1, GSTT1,GSTP1) genes polymorphisms in childhood acute lymphoblastic leukemia

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Glutathione S-transferases (GSTs) are enzymes involved in the detoxification of environmental mutagens, carcinogens and drugs.We investigated the association between GSTM1, GSTT1 and GSTP1 genes polymorphisms and risk of acute lymphoblastic leukemia (ALL). GSTM1 and GSTT1 genotypes exhibit deletion polymorphisms, which in case of homozygosity (GSTM1 0/0, GSTT1 0/0) lead to absence of enzyme activity. GSTP1 gene displays polymorphisms within its coding region at codon 105 (Ile105Val). The coding region polymorphisms within GSTP1 have been suggested to confer different catalytic activities.
We analyzed GST genotypes in 254 children with ALL and 246 control individuals.In this study frequencies of combined GSTM1 0/0 and GSTP Ile/Ile genotypes and combination of GSTT1 1/1 and GSTP1 Ile/Ile were higher among the ALL patients, than control group (30% versus 19%, P = 0.005, 48% versus 32.5%, P = 0.0005, correspondingly). Frequency of combined GSTT1 1\1 and GSTP1-Ile/Val genotypes was elevated significantly in control group (40% versus 26%, P = 0.001). ALL cases were separated by the following criteria: remission and relapse. The frequency of combined GSTM1 1/1 and GSTT1 1/1 genotypes , GSTP1-Ile/Val allele prevailed in children with relapses (56% versus 39%, P = 0.04, 63% versus 33%, P = 0.0003, accordingly), but frequency GSTP1-Ile/Ile allele significantly higher in remission (65% versus 28%, P = 0.0001).We have also found statistically significant differences in distribution of combination GSTT1 0/0 and GSTP1-Ile/Ile genotypes according to sex (elevated on girls, P = 0.01). Overall no associations were found with age at diagnosis and GSTs variants.

P0432. BRAF Mutations and RET/PTC Rearrangements in Papillary Thyroid Carcinoma.

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Rearrangements of the RET proto-oncogene (RET/PTC) and BRAF gene mutations are the major genetic alterations in the etiopathogenesis of papillary thyroid carcinoma (PTC). We have analyzed a series of 118 benign and malignant follicular cell-derived thyroid tumors for RET/PTC rearrangements and BRAF gene mutations. Oncogenic rearrangements of RET proto-oncogene was revealed by semiquantitative RT-PCR of simultaneously generated fragments corresponding to tyrosine kinase (TK) and extracellular RET domains. The clear quantitative shift toward the TK fragment is indicative for the presence of RET rearrangements. The overall frequency of RET/PTC rearrangements in PTC was 14% (12 of 85), including 7 RET/PTC1, 2 RET/PTC3, 1 deltaRFP/RET and 2 apparently uncharacterized rearrangements. The most common T1796A transversion in BRAF gene was detected in 55 of 91 PTC (60%) using mutant-allele-specific PCR. We also identified two additional mutations: the substitution G1753A (E585K) and a case of 12-bp deletion in BRAF exon 15. Moreover, there was no overlap between PTC harboring BRAF and RET/PTC mutations, which altogether were present in 75.8% of cases (69 of 91). Taken together, our observations are consistent with the notion that BRAF mutations appear to be an alternative pathway to oncogenic MAPK activation in PTCs without RET/PTC activation. Neither RET/PTC rearrangements nor BRAF mutations were detected in any of 3 follicular thyroid carcinomas, 11 follicular adenomas and 13 nodular goiters. The high prevalence of BRAF mutations and RET/PTC rearrangements in PTCs and the specificity of these alterations to PTC make them potentially important markers for the preoperative tumor diagnosis.

P0433. Role of a promoter polymorphism in the CYP17 gene in familial aggregation of Prostate Cancer

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The CYP17 gene, encoding the cytochrome P450c17a enzyme, is a likely candidate for prostate cancer because it is directly involved in the production of testosterone. A thymidine to cytosine transition (designated A2 variant) identified in the promoter region of CYP17, has previously been associated with a familial history of prostate cancer in North-American families. The purpose of our study was to determine whether a correlation of the CYP17 A2 allele with familial prostate cancer could be replicated in a European population. An association study was conducted, including 82 unrelated familial prostate cancer probands, 92 prostate cancer probands without affected relatives (sporadic cases) and 88 healthy men from Germany. Genotypes of the restriction fragment length polymorphism in the promoter of CYP17 were assessed by MspAI digestion. Case – control comparisons were performed by modelling a dominant (A1/A2 + A2/A2 vs. A1/A1) and a recessive (A2/A2 vs. A1/A2 + A1/A1) effect of the promoter modification. An insignificant overrepresentation of homozygous carriers of the A2 allele (recessive effect) was found in sporadic prostate cancer cases, as compared to controls (OR = 2.2; 95%CI, 0.96 – 5.0). However the A2 variant was not related to familial disease, neither under the dominant (OR = 0.7; 95%CI, 0.4 – 1.2) nor under the recessive model (OR = 1.0; 95%CI, 0.5 – 1.9). Our results do not suggest a role of CYP17 as a high risk susceptibility gene for familial prostate cancer nor as a modifier for the disease risk in the European population.

P0434. Identification of two novel large genomic deletion in MSH2/MLH1 and evaluation of different screening techniques

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Large genomic rearrangements in the mismatch repair genes MSH2 and MLH1 are estimated to account for up to 27% of all mutations in patients with hereditary nonpolyposis colorectal cancer (HNPCC). Since large genomic deletions are missed by direct DNA sequencing, two novel methods were recently introduced to overcome this problem: i) the quantitative multiplex PCR assay (QMPA), ii) the multiplex ligation dependent probe amplification (MLPA) assay. Whereas the first method divides all 35 exons of MSH2 and MLH1 into 7 separate groups for PCR multiplexing, MLPA amplifies up to 45 sequences simultaneously.
We tested both methods on 4 verified MSH2/MLH1 deletion samples to subsequently investigate a group of 35 Swiss patients clinically suspected of HNPCC, in whom no germline mutation could be identified by direct DNA sequencing. In 13 of 26 (50%) available colorectal cancers microsatellite instability was present, 9 of which showed immunohistochemical loss of either MSH2 or MLH1.
Both techniques, QMPA and MLPA, readily identified the deletions in the control samples. In addition, a novel MLH1 germline deletion spanning exons 7 to 9 as well as a novel MSH2 deletion encompassing exons 7 and 8 were detected. The mutations were found to segregate with disease and were further characterized by RT-PCR and long-range PCR. An additional MSH2 deletion detected by QMPA could not be confirmed by other methods.
In conclusion, we have identified two novel large genomic deletions in MSH2 and MLH1, respectively. Both methods, QMPA and MLPA, appear to be of comparable sensitivity albeit with different specificity.

P0435. Multiprobe interphase cytogenetics delineates two subgroups of multiple mylema

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Multiple Myeloma (MM) is a malignancy of clonal plasma cells accumulating in the bone marrow (BM). Deletions of chromosome 13 and hypodiplody as defined by metaphase cytogenetics confer an inferior prognosis. However, the low proliferation rate limits the analysis on metaphase spreads to ~30%.
We applied interphase-FISH with 8 specific DNA probes for chromosomes 1q21, 6q21, 8p12, 9q34, 11q23, 13q14.3, 17p13, and 22q11 on purified CD138+ BM-cells from 50 newly-diagnosed MM patients. A copy number score (CS) was calculated for each patient by subtracting the number of probes showing losses from the number of probes indicating gains.
Signal changes were detected for a median of 3/8 probes per patient. The most frequent gained signals were found for 9q34 (65%), 11q23 (60%), 1q21 (48%), and 6q21 (21%). Common losses were observed for 13q14.3 (50%), 17p13 (28%), 6q21 (21%), and 8p12 (20%). CS>0 was found in 28/50 and CS≤ 0 in 22/50 patients. Two peaks (CS=-1 and CS=+2) were found by plotting patient numbers over CS values. Monosomy of 13q14.3 was present in 19/22 patients with CS≤ 0, and 6/28 with CS>0 (p=0.0001). Gains of 9q34 and 11q23 were highly associated and more frequent in clones with CS>0 (p=0.0004). Gains of 1q21 as well as deletions of 17p13 and 8p12 were distributed evenly between clones with CS>0 vs. CS≤ 0.
In summary, CS indicates the presence of two distinct MM subgroups, with predominant losses vs. predominant gains. We hypothesize that these correspond to the metaphase-defined non-hyperdiploid and hyperdiploid types of MM, respectively.

P0436. Characterization of polymorphic trinucleotide repeats in genes *TGFBR1* and *RGS19IP1* in various tumors.

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Genes *TGFBR1* and *RGS19IP1* encode proteins that take part in transforming growth factor β (TGF-β) signaling, mainly resulting in inhibition of cell proliferation. *TGFBR1* encodes TGF-β receptor type 1, transmitting signal from TGF-β receptor type 2 to intracellular mediators R-Smads. The 1st exon of *TGFBR1* contains a polymorphic GCG-repeat; $(GCG)_6$ was previously reported as tumor susceptibility allele associated with breast and colorectal cancers. *RGS19IP1* encodes a PDZ domain-containing protein, that interacts with TGF-β receptor type 3 protecting it from degradation and facilitating the receptor interactions. The 5'-untranslated region of *RGS19IP1*contains a CGG-repeat the polymorphism of which hadn't been studied before. We conducted a case-control study on 194 samples of chronic lymphocytic leukemia (CLL), 56 non-small cell lung cancer (NSCLC), 75 breast cancer (BC) and 203 healthy controls. Our BC cases had statistically significant positive association with the $(GCG)_6$-allele (13.8% versus 6.1%, P = 0.016, OR = 2.47 (95%, CI (1.21 - 5.07)), Fisher's exact test, while cases of CLL and NSCLC didn't differ significantly from controls. We have identified a polymorphism of CGG-repeat in *RGS19IP1* with at least 8 alleles: 4 major (CGG_{10-13}) with frequencies 20.4%, 41.6%, 27.6%, 9.4%, respectively, the rest alleles are rare with overall frequency 1% in control; heterozygoty 0,729. Our BC cases had statistically significant positive association with the rare alleles (4.7% versus 1%, P = 0.01, OR = 4.92 (95%, CI 1.42 - 17.1)), NSCLC cases - with CGG_{12} (49.1% versus 27.6%, P = 0.0001, OR = 2.53 (95%, CI 1.65 - 3.9)).

P0437. Genotype-phenotype correlation in patients with hereditary medullary thyroid carcinoma (MTC)

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Germline mutations in the RET-protooncogene predispose to MEN2B, MEN2A and FMTC with a decreased risk and a delay of developing MTC in this line. Patients with FMTC have mainly mutations in exon 10, 13, 14 and 15, while RET mutation of codon 634 (exon 11) is typical for MEN2A.
We compared the phenotype of 73 patients (out of 29 families) with a RET mutation in exons 13-15 (codons 790, 791, 804 and 891) with

66 patients (out of 25 families) with a RET mutation in codon 634. Mean age of the 24 index patients with mutations in exon 13-15 was significant higher compared with the 16 index patients with codon 634 mutation. In the index cases with mutations in exons 13-15 tumour stage at operation was favourable, cure rate was better and the death rate was lower. In the screening group the cure rate was better (93% in patients with mutations in exons13-15, compared to 77% in patients with mutations in exon 11). The natural course of C-cell-carcinoma in patients with germline mutation affecting exons 13, 14 and 15 seems to be less aggressive, presents at higher age and has a reduced penertance as compared to exon 11. A modification of the recommendation concerning age of prophylactic thyroidectomy in RET-mutations exons 13 -15 should be discussed.
* Participants of the MEN 2 Study Group: Höppner, Hamburg; Wahl, Usadel, Happ, Frankfurt; Vogt, Heidemann, Augsburg; Schneyer, Halle; Langer, Saarbrücken; Fuchs-Hammoser, Weiß, Berlin; Varma, Karlsruhe; Schröder, Brandenburg; Hiort, Lübeck; Kummerich, Stuttgart; Meng, Greifswald.

P0438. Low *IFITM1* and high *CXCL3* expression correlate with high-risk chronic myeloid leukemia

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We investigated the peripheral blood gene expression profiles in sixty-seven chronic myeloid leukemia (CML) patients classified according to new prognostic score (NPS). Seven genes were chosen from a preliminary cDNA microarray study in which two high versus two low-risk patients were analyzed. Semi-quantitative and real-time reverse transcription polymerase chain reaction (RT-PCR) analysis of these differentially expressed transcripts highly correlated with the microarray data. Expression levels of all genes, except *PTGS1*, were significantly different between the high (n=9) and low-risk (n=7) CML by semi-quantitative RT-PCR (*IFITM1* and *CXCL3* p=0.001; *CCNH* p=0.012; *RAB1A* p=0.01, *PRKAR2B* p=0.016; *UCP2* p=0.04; and *PTGS1* p=0.315). Real-time RT-PCR analysis showed similar results for *IFITM1* expression in thirty-four low and eleven high-risk patients ($p=9.7976 \times 10^{-11}$). Higher *IFITM1* or lower *CXCL3* expression correlated with improved survival (p=0.01 and p=0.059 respectively). Gene expression profiling is a valuable tool to identify candidate risk group indicator genes for the development of a molecular classification system for CML, which may also predict survival.
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P0439. Molecular characterization of colorectal cancers with loss of PMS2 expression

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Germline mutations in mismatch repair (MMR) genes (*MLH1, MSH2, MSH6, PMS1*, *PMS2*) are responsible for hereditary nonpolyposis colorectal cancer (HNPCC) which account for 2-5% of all colorectal cancers. Mutations in *PMS2* (7p22.2) are relatively rare and thought to account for <5% of HNPCC patients. To date 5 pathogenic *PMS2* mutations have been reported, of which 3 displayed an autosomal dominant and 2 a recessive mode of inheritance.
Out of a consecutive series of 1048 colorectal carcinomas from 5 Swiss hospitals, screened for MMR expression by immunohistochemistry, we investigated 14 patients with colorectal tumours showing selective PMS2 loss for microsatellite instability (MSI) and loss of heterozygosity (LOH) at the *PMS2* locus. Furthermore, we screened all 15 exons of *PMS2* for germline mutations by direct DNA sequencing.
MSI was present in 13 (93%) and consistent LOH in 7 (50%) colorectal tumours. Thus far, we identified 3 novel *PMS2* germline mutations in 6 patients: the mutations 703C>T (exon 6) and 1018delA (exon 10), each are present in one patient, the 1828insA (exon 11) mutation was observed in 4 patients without apparent relationship between the families. Since 2 DNA regions on 7p22.1 and 14q32.31 share more than 90% sequence homology with exon 11 (including flanking introns), we currently confirm the presence of the 1828insA mutation by RT-PCR using unique flanking primers. As DNA sequences highly homologous to *PMS2* may mask mutations, screening the mRNA should further help identifying pathogenic alterations in our patients. To date, we found no evidence for bi-allelic PMS2 mutations/autosomal-recessive inheritance.

P0440. Missense mutations within RING finger domain of BRCA1 gene detected in high risk Czech patients with hereditary breast and ovarian cancer

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Germline mutations in highly penetrant cancer susceptibility gene BRCA1 cause genetic predisposition to breast and ovarian cancers. Mutations generating a premature stop codon are considered to be pathogenic. However, distinguishing disease-causing missense mutations from rare polymorphisms remains problematic. Three different missense mutations located within conserved C_3HC_4 RING finger domain of BRCA1 gene were repeatedly detected in unrelated high-risk Czech breast and/or ovarian cancer families: p.Met18Lys (M18K); p.Cys39Arg (C39R); p.Cys61Gly (C61G). The RING finger motif of BRCA1 gene is involved in protein-protein interactions. The p.Cys61Gly is frequent mutation and was proved by many authors to segregate with the disease in several breast cancer families. However, p.Met18Lys detected in 3 Czech families is not recently present in BIC database and p.Cys39Agr detected in 3 Czech families has been submitted only twice to BIC database. None of these missense mutations was present in our control group of 50 postmenopausal unrelated healthy women without family history of breast cancer. In the poster we present pedigrees of these high-risk breast/ovarian cancer families and summarize all the arguments including structural consequences why we assume that also the p.Met18Lys and p.Cys39Agr mutations are likely to be disease-causing.
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P0441. Chromosomal Band 8p22 and its Contribution to Tumor Formation in Ovarian Cancer

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Chromosomal band 8p22 is frequently affected by chromosomal loss in nearly all solid tumor types. Eight genes from 8p22 were associated with tumor biology with rather poor supporting evidence and functional characterization. None of them is associated with ovarian cancer, although the percentage of loss of heterozygosity is most impressive throughout 8p22. This fact led us to perform a systematic investigation of genes located on 8p22 with respect of ovarian cancer. We collected information about LOH and analyzed the expression pattern of genes using published gene profiling data, and integrated this information with a comprehensive in silico annotation. Finally, we concentrated on eight genes which are reliably expressed in the ovary and either show frequently reduced expression or have interesting proposed functions. We have acquired quantitative real-time PCR data from primary ovarian tumor samples and cell lines. The results provide strong evidence for the involvement of at least two genes (N33, EFA6R) in ovarian cancer biology. Additionally, we identified several ovarian tumor cell lines lacking the expression of these genes. Now, we assess the methylation status

of these two genes, as epigenetic regulation of gene expression may provide us further evidence for their role as tumor suppressor genes. Further analysis of these candidate tumor suppressor genes will include reconstitution experiments in cell lines lacking the expression in combination with xenotransplant experiments. We believe that our systematic approach will provide deeper insights into pathogenesis of ovarian cancer and elucidate the role of the chromosomal band 8p22 in this deadly disease.

P0442. Aberrant gene expression in chronic myeloid leukemia is not associated with recurrent *MECP2* mutations

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Aberrant gene expression in chronic myeloid leukemia (CML) has been linked to epigenetic alterations (methylation of CpG islands, loss of imprinting-LOI) or mutations in the genes that make complexes with histone deacetylase. LOI of insulin-like growth factor-II (*IGF2*) is specifically associated with disease progression independent of hypermethylation of the *H19* CpG island in the accelerated and blastic crisis phases of CML (Randhawa et al., *Blood* **91**, 3144, 1998). Methylation-dependent silencing at *H19* imprinting control region (ICR) is mediated by methyl-CpG-binding protein 2 gene (*MECP2*) (Drewell et al., *Nucl. Acids Res.* **30**, 1139, 2002). Based on these observations we hypothesized that somatic inactivation of *MECP2* may play an important role in disease progression in CML, and investigated *MECP2* gene mutations in the peripheral blood samples collected from 36 CML patients at chronic, accelerated and blastic phases. DNA was extracted and eight mutation hotspots in *MECP2* (R106W, P152R, F155S, T158M, R168X, R270X, V288X, and R306C) were screened by PCR and restriction enzyme digestion. None of the mutations was present in any of the samples investigated, and an association between *MECP2* mutations and CML disease progression was not observed. However, inactivation of *MECP2* by epigenetic alterations cannot be ruled out (Supported by grants from TÜBİTAK-SBAG and Bilkent University Research Fund).

P0443. *BRCA 1* and *BRCA 2*: Breaking the cycle and repairing the damage

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BRCA1 and BRCA 2 proteins are both 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 BRCA 1 and BRCA 2 mutations have a specific phenotypic response to irradiation compared with age matched controls. We have investigated mutation carriers for apoptosis and chromosome breakage in response to irradiation in peripheral blood lymphocytes. Using a BRDU incorporation assay and flow cytometry, we have also assessed the cell cycle dynamics of peripheral blood lymphocytes in BRCA1 and BRCA 2 mutation carriers who have never been diagnosed with cancer,compared with age matched controls.

At present, we have recruited 32 BRCA mutation carriers and controls for these patients. In the first seven BRCA mutation carriers there is limited evidence that there may be a reduction in the repair of chromosome breaks, excluding gaps, between carriers and controls (p=0.05). On average, there are more abnormalities per cell, other than gaps, in the carriers than the controls for each of the six time points (p=0.014).

In the first 29 BRCA mutation carriers there is also evidence that the apoptotic response (apoptotsis at 4Gy - spontaneous apoptosis) may be reduced in peripheral blood lymphocytes compared with controls (p=0.015). Our results of chromosome breakage, apoptotic response and cell cycle dynamics will be discussed in relation to possible mechanisms for cancer susceptibility and cell cycle control.

P0444. The importance of the detection of telomerase activity in patients with chronic lymphocytic and acute myeloblastic leukemia: Our first results

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Telomeres, which are located at the ends of eukaryotic linear chromosomes protect ends of chromosomes against degradation. Telomere lenght is controlled by telomere proteins and telomerase enzyme complex. Telomerase activity is not detectable in somatic cells except for testes, fetal ovary, hematopoietic stem cells, lymphocytes, hair follicles and cryptic cells in the intestine. However high telomerase activity had been detected in %85 of human malignancies. Thus it has thought that telomerase activity is a important marker in the cancer diagnosis.

In our study telomerase activity was determined by using TeloTAAGGG Telomerase PCR ELISA^{PLUS} (Roche) kit in total 21 peripheral blood samples collected from 7 chronic lymphocytic leukemia (CLL), 7 acute myeloblastic leukemia (AML) patients and 7 healthy persons.

As a result, telomerase activity was low in the CLL patients and high in the AML patients according to control group. But the differences between the groups statistically was not significant.

P0445. APC gene variants among patients with familial adenomatous polyposis

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Among 66 probands with familial adenomatous polyposis (FAP) 37 mutations (55%) were found. Seventeen mutations (47%) were revealed for the first case. Mutations 1309del5 and 1061del5 were found recurrent in Russia (25% of all mutations). Because mutations in genes another than APC explains only small percent of FAP without APC mutations we analysed the APC polymorphisms in attempt to find specific the gene variants. Three polymorphisms located within exons of APC and one in 3' untranslated region of the gene were investigated among probands and in control group. These polymorphisms defined 7 haplotypes, two of which were common and five - relatively seldom. The latter may be characterized as a result of the common haplotype rearrangement. Homozygotes on the most frequent haplotype were less frequent among probands without the mutations than in control group (OR=0.37) but this difference was not statistically significant (95% CI: 0.15-1.06). However, the proband group without APC mutations is not homogeneous. It was divided in two subgroups: with the many affected relatives of proband (> 2; the mean quantity 5) and with small quantity of affected relatives (< 2). The frequency of the homozygotes in the latter subgroup was significantly less than in the control group (OR=0.20; 95% CI: 0.02-0.67). The genomes with the seldom haplotypes were more frequent among probands without the mutations than in control individuals (OR=3,38; 95% CI: 1.49-9.22). These results may suggest an association of the APC variants with another gene (that confer increased risk of FAP) or with non-observed APC defects.

P0446. Eukaryotic releasing factor 3 gene expression in gastric cancer

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Gastric cancer is a major cause of cancer-related dead worldwide.

Despite this fact, the knowledge of the molecular events underlying the development of gastric tumors is still poor. In a recent past, several initiation and elongation factors were found to be overexpressed in different kinds of tumors, but any study has been done to investigate the expression patterns of releasing factors. The eukaryotic releasing factor 3 (*eRF3/GSPT1*), which regulates protein synthesis as a GTP-dependent stimulator of eRF1 in termination of translation also has other non-translational roles, namely participating in the mRNA decay machinery, regulating the cell cycle and apoptosis. In this study we analysed the expression pattern of *eRF3/GSPT1* using a quantitative real-time RT-PCR, and we also determined the gene dosage by quantitative real-time PCR in 25 gastric tumors. Different histological types of gastric tumors and adjacent non-neoplasic tissue were used for gene expression analysis. From all the tumors analysed about 35% revealed overexpression of *eRF3/GSPT1*. Moreover, 70% of the intestinal type tumors overexpressed the gene while only 10% of the diffuse type tumors did so. A quantitative real-time PCR was also performed to determine *eRF3/GSPT1* gene dosage, and no amplifications were detected. There was no correlation between *eRF3/GSPT1* expression and gene copy number. We postulate that overexpresson of *eRF3/GSPT1* in gastric adenocarcinomas can be due to the need to increase translation of specific oncogenic transcripts. Alternatively, *eRF3/GSPT1* can be involved in tumurigenesis by its non-translational roles, namely (des)regulating cell cycle, apoptosis or transcription.

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P0447. Analysis of BRCA1 gene mutations in breast cancer patients in Moscow Region of Russia using biochips

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Germ-line mutations in *BRCA1* gene account for a substantial proportion of inherited breast cancers. The mutation frequency of *BRCA1* gene in women with breast cancer varies according to family history, age at diagnosis and ethnicity. In the Russian population, the frequency of different *BRCA1* mutations has not studied thoroughly yet. We have begun the screening of BRCA1 mutations in women of Moscow Region (age 32 to 75) diagnosed with breast cancer and unselected for family history of this disease.

The method of *BRCA1* mutations carriers identification based on hybridization with microarray of gel-immobilized oligonucleotides (biochip) has been developed. Five different mutations: 300T>G, 185delAG, 4153delA, 4158A>G and 5382insC were selected for the analysis.

To date, we have analyzed DNA of 61 women with breast cancer for *BRCA1* gene mutations. 5382insC mutation was identified in three patients with ductal breast cancer (4.9%). The other mutations were not detected.

Our results suggest that BRCA1 mutations in unselected breast cancer in our region likely to be found in high risk families only. We have shown that the gel-based microarray technology provides a rapid and a reliable detection method for the analysis of mutations and will be useful for screening of larger breast cancer populations. The study is currently in progress and the preliminary results will de discussed at the meeting.

P0448. Two recurrent mutations in MSH2 and MLH1 account for 14% of germline mutations detected in a large series of German patients with hereditary non-polyposis colorectal cancer (HNPCC)

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Mutations in DNA MMR genes, mainly MSH2 and MLH1, account for HNPCC, an autosomal dominant cancer predisposition to colorectal cancer and other malignancies. Evaluation of current HNPCC surveillance recommendations or mutation detection requires well characterised HNPCC cohorts of reasonable size. A large HNPCC cohort is currently established by the German HNPCC Consortium. This study presents the spectrum and frequencies of germline mutations in MSH2 and MLH1 in 1425 unrelated HNPCC suspects. Microsatellite analysis in tumor tissue was applied to pre-select patients eligible for mutation analysis.

High microsatellite instability (MSI-H) was detected in 72% of patients meeting the Amsterdam criteria (AC) and in 37% of the patients fulfilling less stringent criteria. 465 patients (406 MSI-H, 11 MSI-low and 48 meeting the AC in whom no tumor samples were available) were screened for mutations. In 134 patients a pathogenic MSH2 mutation and in 118 patients a pathogenic MLH1 mutation was identified. 160 distinct mutations were detected. Of note, MSH2,c.942+3A>T and MLH1,c.1489_1490insC were identified in 11% and 18% of the MSH2 and MLH1 mutation carriers, respectively. Our findings underscore the value of microsatellite analysis as a pre-selection tool in patients not meeting the AC and have implications for mutation detection in Central European HNPCC populations. The large number of mutation carriers detected by our study represents an ideal basis for detection of genetic modifiers or exogenous factors influencing the HNPCC phenotype and evaluation of the current surveillance recommendations.

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P0449. Hereditary non-polyposis colorectal cancer: Pitfalls in deletion screening in MLH1 and MSH2

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Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal-dominant disease, based on germline mutations in DNA mismatch repair genes, mainly in MSH2 and MLH1. About 10% of mutations are large genomic deletions encompassing one or more exons. MLPA (multiplex ligation-dependent probe amplification) is a reliable, easy to perform method for deletion screening in both genes in one single step. Therefore deletion screening was proposed to be performed in patients suspected of HNPCC before further labour-intensive screening for point mutations in MSH2 and MLH1.

During deletion screening in 202 patients by MLPA we detected deletions of one or more exons in 19 patients. In two patients we identified a deletion of exon 13 of the MLH1 gene. Interestingly it could be confirmed by sequencing of cDNA only in one of the patients. Sequencing of genomic and cDNA of the second patient revealed a point mutation that is localized in the hybridization sequence of the MLPA probe of exon 13.

We observed a similar case when we applied deletion screening by fluorescent multiplex PCR: a deletion of MSH2 exon 10 only has been detected in one patient. Sequencing revealed a point mutation in exon 10 that was also localized in the primer sequence.

We conclude that results of the MLPA test (or other methods applied for deletion screening) are usually correctly interpreted when two ore more adjacent exons are concerned. However, when only one exon appears to be deleted it has to be verified by an additional method.

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P0450. IGH/MYC complicons in human IGH/BCL2-positive germinal center B-cell lymphoma

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Oncogene activation via juxtaposition to the *IGH* locus by a chromosomal translocation, or less frequently, genomic amplification, is supposed to be a major mechanism of B-cell lymphomagenesis. However, amplification of an *IGH*/oncogene fusion, recently coined as a complicon, is a rare event in human cancers and has been reported to be associated with poor outcome and resistance to treatment. We observed two cases of clinically aggressive germinal center derived B-cell lymphomas with *IGH/BCL2* fusion, which additionally displayed amplification of an *IGH*/*MYC* fusion. The first case contained the *IGH*/*MYC* complicon in multiple double minutes whereas the second case showed a *BCL2/IGH/MYC* triple fusion complicon on a derivative chromosome 8, as shown by fluorescence in situ hybridization. Additional molecular cytogenetic analyses revealed that the first case also contained a chromosomal translocation affecting the *BCL6* oncogene and a biallelic inactivation of *TP53.* The second case additionally harbored a duplication of *REL* and acquired a translocation affecting *IGL* and a *TP53* deletion during progression. Complicons affecting *IGH/MYC* have been previously reported in lymphomas of mouse models simultaneously deficient for *TP53* and the non-homologous end joining DNA repair pathway. To the best of our knowledge, this is the first time that an *IGH*/*MYC* complicon is reported in human lymphomas. These findings imply that that the two mechanisms resulting in *MYC* deregulation, i.e. translocation and amplification, can occur simultaneously.

P0451. Modelling the MLL-gene translocation in vivo

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MLL-gene translocations are the most common abnormalites occurring in secondary leukaemias resulting from treatment with topoisomerase-II-inhibitors. Several in vitro studies have indicated that the MLL-locus is cleaved as a result of apoptotic processes. This to our knowledge has not yet been studied in vivo.
A normal EBV-transformed cell line was treated with 25µM etoposide, after which 4% of the interphase nuclei showed MLL-cleavage (0.5% in controls). Using MLL-FISH and TUNEL- or cleaved caspase-3-assays, which detect apoptotic cells, we envisioned MLL-cleavage and apoptosis status simultaneously. Only 13% of MLL-split signals occurred in apoptosis positive cells. This showed that the MLL-gene broke in non-apoptotic cells or before apoptosis may have occurred. We induced metaphases by premature chromosome condensation (PCC) after etoposide treatment to test whether MLL-breaks detected in interphase nuclei are indicative of MLL-gene translocations. A total of 137 metaphases were tested for MLL-status on random days after etoposide treatment. Two translocations were found one day and one translocation seven days following treatment. Fourteen days following treatment, only one metaphase in 850 showed an MLL-gene translocation. An additional interphase analysis 50 days after treatment showed four MLL-splits in 1600 treated cells (control: 1 in 2500). Thus, cells containing an MLL-gene translocation following etoposide treatment are rare. They may survive due to a selection process in viable cells and not always undergo apoptosis. Furthermore, even though MLL-gene translocations occur, the MLL-gene is not a preferential target of etoposide, since many cells showed numerous other chromosomal aberrations when analysed with SKY.

P0452. Chromosomal rearrangements affecting the BCL6 and MYC loci are rare events in classical Hodgkin lymphoma

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Hodgkin lymphoma (HL) is a peculiar B-cell malignancy in which the tumor cells only represent 0.1-1% of the affected tissue. HL is subdivided into classical (cHL) and nodular lymphocyte predominant (NLPHL) Hodgkin lymphoma. The tumor cells of cHL, called Hodgkin/Reed-Sternberg (HRS) cells, express CD15 and CD30, whereas the tumor cells of NLPHL express B-cell markers like CD20. Chromosomal alterations involving the *BCL6* and *MYC* loci are among the most frequent genomic changes in various B-cell malignancies. In cHL, despite its predominant B-cell origin, the presence of chromosomal changes involving *BCL6* and *MYC* has not yet been studied. Here, we have evaluated 32 cHL by FISH applying probes flanking the *BCL6* and *MYC* loci. Only large hyperploid nuclei suggestive for HRS cells were scored. Signal patterns pointing to translocations targeting *MYC* were not observed in any of the 28 cHL evaluable. With regard to numerical changes, a median number of 3 copies of *MYC* (range 3-8) was observed. One cHL displayed a high-level *MYC* amplification. Translocations affecting *BCL6* were detected in 2/32 (6%) cHL. The median number of *BCL6* copies was 4 (range: 2-5). Additionally, cryosections of 14 NLPHL were studied by combined immunofluorescence for CD20 and FISH for *BCL6*. Breakpoints affecting the *BCL6* locus were detected in 5 cases (36%) suggesting a significantly higher incidence in NLPHL than in cHL (2/32 vs. 5/14; p=0.02). Our results indicate that rearrangements of *BCL6* and *MYC* are less frequent in cHL than in other B-cell lymphomas.
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P0453. Molecular cytogenetic breakpoint mapping of a reciprocal balanced translocation (11;21)(q23.3;q11.2) in bone marrow cells of two patients with myelodysplastic syndrome

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Myelodysplastic syndrome (MDS) is a clonal disorder of haematopoietic stem cells characterized by cytopenia and bone marrow dysplasia that mostly affects elderly persons and frequently evolves to acute myeloid leukemia (AML). Loss of genetic material such as -5/del(5q) and -7/del(7q) is frequently associated with primary MDS, reciprocal translocations are less common. Here, we present data on two patients with MDS with a rare recurrent translocation t(11;21)(q23.3;q11.2). A previous molecular cytogenetic study in one case with t(11;21) mapped the translocation breakpoints to a genomic area defined by two flanking YAC clones in 11q23.3 and within a 800 kb region in 21q11.2 (Wlodarska et al., 1999). Based on these data the aim of the present study was to identify breakpoint spanning clones by FISH using publicly available PAC and BAC resources (Ensemble, UCSC, NCBI) in order to identify candidate genes disrupted or deregulated by this translocation. FISH with selected clones resulted in the identification of one breakpoint spanning clone in 21q11.2 and one deleted clone in 11q23.3. Clones from the critical regions might also be involved in cryptic rearrangements and will be tested subsequently on metaphase spreads of MDS patients with normal karyotypes.(Supported by the Austrian Cancer Society/Tirol)

P0454. A genome wide linkage search for prostate cancer susceptibility genes in families from Germany

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Prostate cancer is a complex disease with a substantial genetic

contribution involved in the disease risk. Several genome wide linkage studies conducted so far have demonstrated a strong heterogeneity of susceptibility loci. In order to assess candidate regions which are particularly relevant for our population, we applied a genome wide linkage approach on 139 German prostate cancer families, representing 405 affected men. Out of these, 309 cases as well as 116 healthy relatives were genotyped at 500 markers (panel P1400, deCODE) with an average spacing of 7.25 cM. We used GENEHUNTERplus to calculate nonparametric Zlr-Scores in the entire family sample and in subsets. In our unselected family collection linkage was most evident at 8p22 (Zlr = 2.47), close to the previously identified susceptibility gene MSR1. Further local maxima with Zlr > 2 were observed at 1q, 5q and 15q. In a subgroup of 47 families which matched the Johns Hopkins criteria of hereditary prostate cancer, suggestive linkage was found at a previously undescribed region on 1p31 (Zlr = 3.37). The remaining 92 pedigrees without a strong disease history yielded a maximum Zlr = 3.15 at 8q13, possibly indicating a gene with reduced penetrance or recessive inheritance. Our results show that predisposition to prostate cancer in Europe may be similarly heterogeneous as demonstrated already for North American populations. In Germany, the MSR1 gene could play a significant role. Other conspicuous loci, like 1p31 and 8q13, would need further investigation in order to verify their relevance and to identify candidate genes.

P0455. The BRCA1 and BRCA2 association among patients with sporadic and familial breast cancer without the genes mutations

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Under investigation of familial breast cancer we found 36% of probands with BRCA1 and BRCA2 mutations (32% and 4%, respectively) in our sample of Russian families. The hypothetical additional gene BRCAX that could explain the rest part of the families is not found up to now. With the aim to clarify possible role of BRCA1 and BRCA2 gene variants as low penetrance genes we analyzed a combination of frequent polymorphisms of these genes in population-based series of breast cancer patients (n=94) in comparison with control sample (n=109).

SNPs analysis has defined two major haplotypes of BRCA1 gene: consensus sequence (haplotype A) and sequence with a set of SNPs in strong linkage disequilibrium (haplotype B; frequency equals 0.36). The BRCA2 contains frequent polymorphism N372H (frequency equals 0.22). We revealed that the haplotype B of the BRCA1 gene is associated with the variants 372H of the BRCA2 not by chance among patients (P=0.033). There was no association between these haplotypes in genomes of control individuals. The frequency of the haplotype B - 372H combination was significantly higher among the patients in comparison with the control individuals (odds ratio: [[Unsupported Character - О]]R=1,93; [[Unsupported Character - Р]] = 0,039). It is interesting that the same phenomenon was observed on the sample of probands with familial breast cancer without BRCA1/2 mutations. The odds ratio for haplotype B - 372H combination among the probands was equal to 3,29 (95% CI: 1,19 - 8,51), that is higher than among the patients with sporadic breast cancer.

P0456. Analysis of Association Between Glutathione S-Transferase M1 and T1 Polymorphisms and Laryngeal Squamous Cell Carcinoma: A Case-Control Study

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Glutathione S-transferases (GSTs) are a family of cytosolic or microsomal enzymes that catalyze a number of reduced glutathione (GSH)-dependent reactions. This ability to metabolize reactive metabolites of carcinogens has been related to a regulating susceptibility to cancer. Interindividual variation in GST polymorphisms has been associated with differences in risk, with respect to several types of cancer. Laryngeal squamous cell carcinoma (LSCC) is one of the five most common cancers worldwide. Epidemiological studies suggested that the etiology of LSCC is multifactorial such as smoking, alcohol consumption, viral infection, and genetic factors. Although smoking and alcohol consumption play a major role in the etiology of LSCC, only a fraction of smokers and drinkers suffers from this disease, suggesting a genetic susceptibility in the general population. Therefore, we conducted a case-control study to analyze the genetic susceptibility of LSCC in patients and investigated the gene polymorphism of the two enzymes; *GST-M1* and *GST-T1.* In the present study, the primary findings of allelic distribution *GST-M1* and *GST-T1* in the patient group and a cancer-free control were determined, and the association between the *GST-M1* and *GST-T1* polymorphisms and LSCC were evaluated.

P0457. Analysis of the 3D-chromatin architecture of the *MLL*-gene region within the chromosome 11 territory and in relation to its most frequently found translocation partner chromosomes

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It is poorly understood why chromosome translocations are recurrently found at specific tumor translocation breakpoints (TBP) in the genome of cancer cells. The region of the mixed-lineage leukaemia (*MLL*-) gene on 11q23.3 represents such a TBP frequently showing translocations with specific partner chromosomes in acute leukemias.

We hypothesize that higher-order spatial genome organization might be a contributing factor in the formation of *MLL*-gene translocations. Therefore we are testing if the *MLL*-region shows a preferential spatial localization within the nucleus in particular in relation to the territory of chromosome 11 as well as to chromosomes 4, 9, and 19 representing the most frequently found translocation partners and, as a control, to chromosome 3 that is not found as a translocation partner.

Experiments include a) hybridizations of one BAC-clone covering the *MLL*-gene in combination with paints for chromosomes 3, 4, 9, 11, and 19 to normal human fibroblasts, lymphoblastoid cells, and possible *MLL*-precursor cells with highly preserved *in-vivo* spatial chromatin architecture using 3D-FISH, b) analysis of the hybridizations with 5-color confocal microscopy, and c) comparison of the frequencies of experimentally determined chromosome vicinities with vicinities found in simulated cell nuclei with random distribution of chromosome territories.

Preliminary results obtained in normal human fibroblasts and lymphoblastoid cells do not indicate preferential vicinities of chromosomes frequently involved in *MLL*-gene translocations, but it appears to exist a preferential localization of the *MLL*-locus in the periphery of the chromosome 11 territory.

P0458. Tissue microarray analysis of cyclin D1 gene amplifications and gains in colorectal carcinomas

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Colorectal cancer is one of the most common neoplastic diseases and one of the leading causes of cancer-related deaths. There are two forms of the disease - hereditary and sporadic. Sporadic colorectal cancer is the most frequent, predominantly affecting individuals of over 70-75 years of age.

Elevated beta-catenin levels in colorectal cancer caused by mutations in beta-catenin or in the adenomatous polyposis coli molecule gene (APC) result in the binding of beta-catenin to LEF-1 and increased transcriptional activation of mostly unknown target genes. CCND1 gene is a direct target for transactivation by the beta-catenin/LEF-1

pathway through a LEF-1 binding site in its promoter region.
CCND1 amplification is the main cause for protein overexpression in numerous human carcinomas such as breast carcinomas, squamous cell carcinomas of the head and neck, epithelial ovarian tumors and bladder cancers. In colorectal cancer, no CCND1 amplification was reported so far.
The aim of the study was to determine the frequency of CCND1 amplifications and gains in a large number of colorectal carcinomas, arranged in a tissue microarray, in order to assess their role in the colorectal cancer development.
Increased gene copy number was found in 42 out of 386 tumors (10.1%). The copy number changes were predominantly gains (7.6%) and much more rarely amplifications (2.5%). No association was established between the copy number changes and the tumor stage, grade or localization.
We concluded, that even in a small group of colorectal tumors, CCND1 gene amplification is a possible mechanism for cyclin D1 oncoprotein increased level.

P0459. Relevance of RNASEL mutations for prostate cancer predisposition in Germany

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Germline mutations predisposing to prostate cancer have been found in three candidate genes so far: ELAC2 at 17p11, RNASEL at 1q25 and MSR1 at 8p22. RNASEL, encoding the 2´,5´-oligoadenylate-dependant RNase L, seems to have rare founder mutations in different ethnicities, like M1I in Afro-Americans, E273X in men of European descent and 471delAAAG in Ashkenazi Jews. In order to evaluate the relevance of this gene in the German population we determined the spectrum and frequency of RNASEL germline mutations by sequencing of the coding exons 2 - 7. This screening included 325 affected men from 145 Caucasian families, of which 48 met the Johns Hopkins criteria for hereditary prostate cancer. In addition, we used 230 non-familial prostate cancer patients and 209 healthy, elderly men as controls. Sequencing revealed only two sib pairs (1.4% of our families) segregating conspicuous RNASEL variants with the disease, the nonsense mutation E273X and a new amino acid substitution (R400P) of unknown functional relevance. Both alleles were also found in our sample of healthy men, with 1.4% and 0.5%, respectively. The common polymorphisms I97L, R462Q and D541E were observed with equal frequencies among prostate cancer families, non-familial patients and controls.
In contrast previous studies we did not find evidence that common variants (i.e.R462Q) may modify disease risk. Moreover, our results are not consistent with high penetrance of deleterious RNASEL mutations. According to the low frequency of germline mutations present in our sample, RNASEL may not have a significant impact on prostate cancer susceptibility in the German population.

P0460. Comparative genomic hybridization (CGH-) analysis of microdissected early and late stage Kaposi's sarcoma (KS)

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Dramatic increase in AIDS-related KS (AKS) has been seen in association with the HIV-AIDS epidemic that in affected patients usually is accompanied with various lethal infections and secondary tumors. Other forms of KS comprise iatrogenic KS (IKS) of immunosuppressed transplantation patients, sporadic KS (SKS) of Mediterranean males, and endemic KS (EKS) found in Equatorial Africa.
Besides the involvement of human herpesvirus 8 that is associated with all forms of KS, the biological nature of KS is still largely unknown and it is not clear whether the disease represents a reactive hyperplastic process or a true clonal sarcoma.
The aim of our study is to do a comprehensive DNA copy number analysis of early patch-plaque as well as late nodular stages of AKS and EKS using microdissection of tumor areas, global DNA amplification, and CGH in 1 patch EKS, 9 nodular EKS, 8 patch AKS, and 11 nodular AKS cases.
Preliminary results show no aberrations in early lesions and sporadic non-recurrent copy number changes in small chromosome segments in late stages. Only one nodular EKS case shows gross losses of whole chromosomes 1, 2, 4, 13, 15, and 16. Lack of gains and losses in early lesions supports the idea that KS initially represents an reactive lesion.

P0461. Analysis of the Mitotic-arrest-deficient-1(MAD1) genotype profiles in a cohort of primary breast cancers, reduction mastectomy specimens and normal blood samples.

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The faithful duplication and separation of genetic material into two daughter cells during mitosis is crucial to the task of cell division. This task is in part monitored by the mitotic assembly checkpoint system (MSC) involving at least 8 different proteins. Two important components of this checkpoint are the resultant proteins of the mitotic-arrest-deficiency-1 (MAD1) gene located at 7p22 in humans *(Jin et al, 1999)* and the MAD2 gene at 4q27 *(Krishnan et al, 1998)*. This interaction occurs by means of two leucine zipper motifs. Importantly, a single nucleotide polymorphism (SNP) at exon 17 of MAD1 within a 2nd leucine motif results in a nucleotide change from arginine[R] (Arg-588 R) to histidine [H] (His-588 H) resulting in a decreased efficiency of hsMAD1 binding to hsMAD2 *(Iwanaga et al, 2002)* . The objectives of the current study was to determine the frequency of R/R, R/H and H/H alleles by genotyping the MAD1 exon 17 SNP in a prospectively collected cohort of breast tumours (n=96), reduction mastectomy specimens (n=15) and blood samples from a normal population (n=15). The methodology involved DNA extraction from fresh frozen tissue, RFLP analysis using *AccII/BstU* restriction enzyme and 6% PAGE analysis. This technique identified that 6/15 (40%) of reduction mastectomy specimens, 4/15(27%) of normal blood samples and 19/96 (20%) of tumour samples displayed the correct R allele. In the clinical setting, functioning of this system may compromise the efficacy of microtubule inhibitors, such as, taxol and taxotere which are increasingly being used in the metastatic breast cancer.

P0462. Detection of CCND1 and ZNF217 copy number changes in ovarian tumors - tissue microarray analysis

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Literature data for the occurrence and significance of CCND1 and ZNF217 copy number changes in ovarian tumors are insufficient. The objective of this study was to asses these alterations in a large number of ovarian tumors and its relation to the tumor phenotype - malignancy, histological type, tumor stage and grade. We applied FISH on a tissue microarrays, containing 900 ovarian tumors - benign, low malignant potential and malignant.
CCND1 amplification was found in 8.46% of malignant, in 8.11% of low malignant potential and was not found in benign ovarian tumors. CCND1 amplification was associated with mucinous type of ovarian cancer (p<0.0001).CCND1 genetic gain was revealed in 9.06% of malignant, in 2.70% of low malignant potential and in 4.87% of benign ovarian tumors. CCND1 gains and amplifications were not associated with the tumor grade and stage.
The frequency of ZNF217 alterations in carcinomas was 25.50% (10.74% gains and 14.76% amplifications). There was not statistically significant difference between the frequencies of ZNF217 copy number changes in different grade tumors. The frequency of gains and amplifications increased significantly from stage I to stage II to stage III tumors. Our results showed association between increases ZNF217 copies and advanced tumor stage. We concluded that genetic alterations in ZNF217 are of prognostic significance for stage progression of the ovarian cancer.

P0463. Molecular analyses of the gene encoding the methyltransferase SUV39H1 in mantle cell lymphomas

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The human *SUV39H1* gene encodes a methylating enzyme, which maintains H3-K9 trimethylation at pericentric heterochromatin and plays a dominant role in heterochromatin organization and chromosome segregation. Double knockout mice of the murine homologues *Suv39h1/h2,* display a dramatic genome instability, and are predisposed to late-onset B-cell lymphomas. *SUV39H1* is located on human chromosome X, which has been suggested to harbor a gene associated with predisposition to mantle cell lymphomas (MCL). This subtype of lymphoma is characterized by a marked male predominance with a male to female ratio of up to 7:1. Its function and genomic location render *SUV39H1* a good candidate associated with predisposition of human MCL. Thus, a total of 24 DNA samples (12 males, 10 females, and 2 male cell lines) from t(11;14)-positive mantle cell lymphomas was screened by SSCP and DHPLC for mutations in the six coding exons and the promotor region of *SUV39H1*. No mutations affecting the amino acid composition or transcription factor binding sites were detected. Moreover, in three SNPs located within the *SUV39H1* locus, the allele frequencies were comparable in MCL cases and healthy controls. The MCL samples were also studied for gross deletions of the *SUV39H1*-locus by fluorescence in situ hybridization (FISH), but only one case out of 24 displayed a chromosomal deletion in the tumor cells. In summary, the present study failed to detect an association of genetic changes of *SUV39H1* and the development of mantle cell lymphomas.
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P0464. Deletions in the von Hippel-Lindau Tumorsuppressor Gene detected by MLPA

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Von Hippel-Lindau disease (VHL) is a tumor susceptibility syndrome based on germline mutations in the *VHL* tumor suppressor gene (chromosome 3p25-p26). It is characterized by a variety of benign and malignant tumors, including renal cell carcinomas, cerebellar and retinal hemangioblastomas, pheochromocytomas and other tumors and cysts.
More than 270 mutations in *VHL* are identified so far. About 80% of the mutations are smaller alterations usually identified by sequence analysis. However, large genomic and intragenic deletions necessitates quantitative southern blotting or fluorescence in situ hybridization (FISH).
Here we report the results of a deletion screening in VHL-patients. This study was performed by applying multiplex ligation-dependent probe amplification (MLPA). MLPA is a recently developed technique which enables, in contrast to the southern-blot technique, the detection of deletions or duplications of single exons relatively fast and in small amounts of DNA. With this method we could clarify the meaning of exceptional sequencing results in a VHL-family. By sequencing exon 1 of the index patient the alteration P25L was demonstrated to appear in a homozygous state. A larger deletion could be excluded by FISH analysis. Sequencing data revealed the mutation heterozygous in the healthy father whereas the mutation analysis of the affected mother revealed no indication for the existence of a mutation. Applying MLPA analysis a deletion of exon 1 and 2 was found in both, the index patient and his mother.
The results of our screening study indicate that MLPA is a sensitive and useful method for the molecular genetic diagnostic of VHL.

P0465. Molecular refinement of the breakpoints in a Wilms'Tumor cell line with a translocation t(7;12)(p22;q22)

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The WT1 tumor suppressor gene at 11p13 encodes a zinc-finger transcription factor that is required for proper development of the kidney with a major role in the epithelial transition of the metanephric blastema. Mutations in the WT1 gene are associated with Wilms' Tumor (WT), a childhood renal malignancy, but are found in only 5-10% of sporadic cases. It therefore has become apparent that several genetic events contribute to Wilms' tumorigenesis. WT1 mutations in premalignant lesions of WTs (i.e. nephrogenic rests), suggest additional genes involved in the WT1-initiated pathogenesis of WT. We previously presented data on a one-year old girl affected with bilateral WT without any other symptoms. We identified a heterozygous germline WT1 nonsense mutation in exon 2, creating a truncated protein of 165 amino acids. In addition, tumor-specific 11p LOH was detected by microsatellite analysis. Cytogenetic studies revealed a seemingly balanced chromosomal translocation t(7;12)(p22;q22) in tumor tissue. We established a cell line from tumor tissue and applied FISH with PAC clones as well as polymorphic microsatellite marker analysis for closer molecular characterization. We isolated a PAC clone overlapping the translocation breakpoint on chromosome 12, and identified a 1,5 Mb deletion on derivative chromosome 7p. This deletion is flanked by the markers sWSS1035 (centromer) and sWSS1796 (telomer), affecting chromosomal bands 7p21.3-7p22.1.

P0466. Mutation analysis of candidate genes for chordoma development

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Previous studies pointed out a correlation between 1p36.13 and development of chordoma, a rare embryogenetic neoplasm arising from notochord remnants.
LOH analysis allowed us to observe LOH in 29/33 tumors, 25 of which sharing a minimal LOH interval between D1S436 and D1S2826 markers.
We applied RT-PCR on 12 chordomas to investigate the role of CASP9, EPHA2, PAX7, DAN and DVL1 genes, selected as candidates on the basis of their mapping in LOH region and of their plausible tumor-suppressor function. We observed DAN, DVL1 and PAX7 specific transcripts in all the chordomas analyzed, while we didn't detect CASP9 cDNA in one sample. We also found that one tumor lacks the extracellular region of EPHA2, while intracellular EPHA2 specific-transcripts were observed in all samples. To search for inactivating mutation/s on candidate genes, we started sequence analysis of EPHA2 and CASP9 cDNAs. EPHA2 study allowed us to identify 1) a novel G->A substitution in a patient constitutionally heterozygous for G/A alleles and emizygous for the A allele in tumor DNA, predicting an Arg -> His substitution; 2) a novel C->T synonymous substitution in a patient constitutionally heterozygous for C/T alleles, predicting a change in mRNA folding. The CASP9 cDNA analysis allowed us to detect in 5/7 patients two reported SNPs, both present in the mRNA and transcribed from the same allele, one of which predicting an Arg-> Gln substitution and a change in mRNA folding. Additional studies are in progress to elucidate the pathogenetic role, if any, of the above sequence alterations.

P0467. $CD3^{-}CD4^{+}$ T cells from two hypereosinophilic patients : characterization of their chromosomal aberrations and gene expression profiling

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The lymphocytic variant of the hypereosinophilic syndrome (LV-HES) is defined by a monoclonal T cell population with increased IL5 production *in vitro*, which is thought to provoke eosinophil expansion *in vivo*. We are currently studying a group of LV-HES patients that are characterized by an abnormal $CD3^{-}CD4^{+}$ T lymphocyte clone. We identified three cytogenetic subclones in fresh blood

from patient P3 using FISH : (6q-): 46,XX,del(6)(q13q22.2) ; (10p-) : 46,XX,del(10)(p11.1p13) ; (6q-10p-) : 46,XX,del(6)(q11.1q23.1), del(10)(p11.1p13). In patient P4, we also detected a 6q- subclone del(6)(q13q22.31), thus delineating the region 6q13q22.2 as commonly deleted in both patients. A retrospective interphase FISH analysis revealed that the 6q abnormalities occured early and were present throughout the chronic phase of disease in P3 and P4. The 6q- subclone emerged predominantly during P3's clinical progression to a T cell lymphoma, representing 92% of the $CD3^-CD4^+$ T cell population at this stage. We postulate that this genetic abnormality reflects the pre-malignant nature of LV-HES and thus are interested in identifying the target gene(s) associated with the 6q-deletion. Using Affymetrix microarrays (the U133A chip, containing 22,000 genes), we have assessed gene expression in purified $CD3^-CD4^+$ T cells from both patients P3 and P4 and compared these to normal $CD3^+CD4^+$ T cells from P4. Genes located in the 6q13-q22.2 region as well as those involved in cell proliferation were found to be underexpressed in the $CD3^-CD4^+$ T cells. They include : p53-regulated PA26 protein, caspase 8 associated protein 2, CD164 and others are currently being investigated further.

P0468. Copy number changes of different loci in a tissue microarray of bladder carcinomas

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Bladder cancer is the fifth most common cancer in Europe. Minimally invasive (pT1) and muscle invasive (pT2-4) tumors show remarkable genetic similarities although the prognosis is definitely worse in pT2-4 tumors.
The aim of our study was to analyze the copy number changes of 9p21, erbB-1, erbB-2, 11q13 (CCND1, FGF4/3, FGF3, EMS1), CMYC and 20q13 in the invasive bladder cancer. A tissue microarray (TMA), containing 159 transitional cell bladder tumor samples, were analyzed by FISH for each of these molecular targets.
FISH revealed decreased copy number of 9p21 in 31.5% of the tumors. The frequency was lower in pT-2 than in pT1 tumors, concerning both homozygous and heterozygous deletions. Amplification of erbB-1 was detected in 4.6% of the tumors, all being muscle invasive. The frequency of erbB-2 amplification increased from pT1 to pT2-4 (4.5% vs 6.7%). 11q13 amplification was found in 4.2% of the tumors. Gains were more frequent than amplifications (9.2%). CMYC amplifications were detected in 1.6% of the tumors and gains in another 9.5%. Gains were associated with the tumor grade but not with the tumor stage. With regard to 20q13, amplification was recorded in 5.3% and gains in 9.6% of the tumors. Gains were associated with the muscle invasion ($p<0.05$) and with the tumor grade ($p<0.001$).
In conclusion, only erbB-1 and 20q13 showed relation to tumor muscle invasion.

P0469. BAC-Array-CGH screening for submicroscopic deletions and duplications at the tumor specific translocation breakpoint 11q23.3 and the nearest common Fragile Site FRA11J

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The array-CGH-technology raises the power of detecting chromosomal gains and losses in patient material from 5-10 Mb offered by conventional CGH to a resolution of 1 - 2 Mb using an array with approx. 2,500 BAC clones (Snijders et al. 2001). In collaboration with G. Meijer and B.Ylstra we established an array with approx. 6,000 clones resulting in a resolution of less than 1Mb. Furthermore, we covered a 7 Mb region on chromosomal band 11q23 with a tiling-path-array.
We tested the hypothesis whether the common Fragile Site (cFS) FRA11J (11q23) is a "hot spot" for DNA double-strand breaks and non-homologous recombination events in 48 acute leukemias showing an MLL-gene translocation (11q23.3). For this purpose we determined the precise location of FRA11J. Using array-CGH we identified DNA gains and losses in the MLL-leukemias with a resolution of less than 1 Mb and screened for genetic instability around both the MLL-gene-breakpoint and FRA11J with a resolution of 100kb. Our data demonstrate a stable genome at the MLL-gene translocation breakpoint and the nearest neighboring cFS FRA11J.

P0470. TP53 mutations in Barrett's oesophagus

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Barrett's oesophagus (BE) is characterised by the presence of metaplastic columnar epithelium containing goblet cells which replaces the normal squamous mucosa. The most important clinical aspect of BE is the increased possibility of development of oesophageal adenocarcinoma. It is thought that this adenocarcinoma develops as a result of progression from a metaplastic columnar epithelium via increasing degrees of dysplasia to an invasive carcinoma. Not all patients with BE develop cancer. Because of difficulty of predicting this risk, all patiensts undergo endoscopic biopsy surveillance for early detection of cancer. Better genetic characterization of risk factors could help the clinicians to select patients with higher risk for increased surveillance. In search for potential prognostic markers, we analysed Barrett's oesophagus tissues and adenocarcinomas in the terrain of BE for TP53 gene mutations. A total of 30 endoscopic biopsy specimens from 27 patients were histologically classified by a pathologist into one of the following groups: 1) BE with metaplasia only (16 samples from 15 patients), 2) low grade dysplasia (LGD) (0 samples), 3) high grade dysplasia (HGD) (2 samples), 4) adenocarcinoma in BE (12 samples). Exons 5 to 9 of the TP53 gene were sequenced. Our results show that while 4 of 12 carcinoma samples tested (30%) contained a TP53 mutation, none of 16 samples from Barrett's oesophagus and none of 2 samples from BE with HGD was mutated. This observation may suggest that TP53 gene mutation may be a relatively late event in the progression from Barrett's oesophagus to adenocarcinoma of oesophagus.

P0471. B-cell malignancies with a t(14;19)(q32;q13) involving the BCL3 locus predominantly derive from IgVH unmutated precursors

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The t(14;19)(q32;q13) translocation juxtaposing *BCL3* in 19q13 with *IGH* in 14q32 is a recurrent chromosomal change in B-cell malignancies, particularly B-CLL. Recently, two types of B-CLL with distinct prognosis have been described according to the presence (favorable outcome) or absence (unfavorable outcome) of somatic mutations of the immunoglobulin heavy chain variable (IgVH) genes. The clinical outcome of patients with t(14;19) is usually poor, and so far, their IgVH mutation status has not been investigated. We established a novel break apart FISH assay for the detection of *BCL3* chromosomal rearrangements. After validation in normal controls,

34 B-cell lymphoproliferative disorders with cytogenetically-proven t(14;19)(q32;q13) were studied by FISH. In 33 cases, involvement the *BCL3* locus was confirmed. The IgVH mutation status was investigated in 24 of these 33 B-cell lymphomas (16 B-CLL and 8 non-Hodgkin lymphomas). In 19 cases (79%), IgVH sequences showed 98.1% to 100% homology to germline VH-genes and, thus, by definition lacked somatic mutations. In the remaining 5 cases, the homology to germline IgVH ranged from 92.6% to 97.8% indicating the presence of somatic hypermutation. These results suggest that tumor cells in cases with t(14;19) predominantly belong to the IgVH unmutated group, which might account for the poor prognosis of patients with t(14;19). As *IGH* breakpoints in t(14;19) mostly involve switch regions, the observation of frequently unmutated IgVH genes suggests that most of the t(14;19) result from an error in class switching during T-cell independent immune responses.
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P0472. Investigation of alterations in fanconi anemia FANCE gene in human breast cancer

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Breast cancer in western world has a high population frequency of about 10% in females. Only a small fraction of it manifests clustered in families mainly due to germline mutations in the BRCA1 and BRCA2 genes. The rest 95% of the breast cancers occur spontaneously as somatic mutations of probably polygenic origin and the underlying gene defects and mechanisms are not clear. Likely candidates are defects in processes of cell cycle, DNA repair, and apoptosis including the genes whose products interact or interfere with DNA repair functions of BRCA1 and BRCA2 Proteins. Recently a novel Fanconi anemia/BRCA pathway has been elucidated which shows five FA proteins (A, C, E, F, G) interacting with BRCA proteins in a common signalling pathway involved in DNA repair. In order to evaluate the contribution of alterations in the FA genes we started screening these genes at genomic and expression level in our collective of breast tumors. In this report we present the first results of mutation screening of FANCE gene.
In a subcollective of 40 breast cancers and 5 breast cancer cell lines without mutations in BRCA genes all exons and intron exon boundaries of FANCE gene were investigated by PCR amplification, SSCP and diret sequencing. Only one mutation in exon 5 has been found. This would argue that mutations in FANCE gene are unlikely to be involved as a frequent cause of defects in the Fanconi Anemia/ BRCA pathway in breast cancer.

P0473. DNA-polymorphisms of NAT2 and MnSOD and predisposition to breast cancer.

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Breast cancer is one of the major cancers around the world (ad 20% of incidence of cancer) but its etiology is still not well understood. Only about 50% of the disease are associated with known risk factors including high-penetrance genes and lifestyle factors. Candidate low-penetrance genes are involved in a variety of pathways, for example, DNA damage by free radicals. The enzymes involved in this mechanism are N-acetyltransferase2 (NAT2) and manganese superoxide dismutase (MnSOD). NAT2 catalyzes acetylation of aromatic amines and hydrasines and forms of free radicals, and MnSOD catalyzes their dismutation. We have assessed the frequency of frequent allelic variants of NAT2 (NAT2*4 (wild type), NAT2*5 (T341C), NAT2*6 (G590A), NAT2*7 (G857A)) in 123 breast cancer cases and 121 controls. We have found allele NAT2*11 in Russian sample as well. Our breast cancer cases had statistically significant positive association with NAT2*6/*6 or NAT2*11 (33,4% vs. 11,0%; P=0,0005; OR=3,06 (95%CI 1,62-5,77) (cases vs. controls)). The frequency of NAT2*5 and NAT2*7 alleles was not significantly elevated in our breast cancer sample compared with controls.
MnSOD gene was studied for polymorphism of valine (V) vs. alanine (A) in the leader peptide at position 16. V/V genotype (MroNI-/-) was associated with decreased risk of breast cancer (24,4% vs. 38,0%; P=0,0268; OR=0,53 (95%CI 0,03-0,91)). The risk of breast cancer decreased at a combination of NAT2*4, NAT2*5 or NAT2*7 alleles and V/V genotype MnSOD (20,3% vs. 36,1%; P=0,0068; OR=0,45 (95%CI 0,25-0,79)).

P0474. Der(Y)t(Y;1)(q12;q12~21) in hematological malignancies : A new case report and review.

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Translocations involving Y chromosome are rare in haematological malignancies. A der(Y)t(Y;1)(q12;q12~21) has been already described in myelodysplastic syndromes and myeloproliferative disorders.
We report a case of Burkitt's cell acute leukaemia with an atypical association t(8;14)(q24;q32) and der(Y)t (Y;1)(q12;q12~21) in a 25 month infant.
Cytogenetic studies revealed clonal abnormalities : 46, XY, dup(1)(q12~21qter), t(8 ;14)(q24 ;q32) [5] /46, X, der(Y)t(Y ;1)(q12 ; q12~21), t(8 ;14)(q24 ;q32) [29] / 46, XY[6].
FISH technique was realized on interphase nuclei using C-MYC probe and showed a c-myc rearrangement. On chromosomal preparations, using Y alpha satellite probe and chromosome 1 WCP simultaneously, we observed three clones : one clone with two chromosomes 1 and a chromosome Y, one clone with one normal chromosome 1, one duplicated chromosome 1 and a chromosome Y and one clone with two chromosomes 1 and a der(Y) t(Y ;1).
In the review of literature, 13 cases only of hematological malignancies with der(Y)t(Y;1)(q12;q12~21) had been reported to date.
Our case is the first case in literature showing a clonal evolution from a dup(1)(q12~21qter), which represents an intermediate step, to a der(Y)t(Y;1)(q12;q12~21) in Burkitt's cell with a c-myc rearrangement. The fact that this translocation has been described in myeloid and lymphoid malignancies suggests that a pluripotent stem cell is affected at the beginning of pathogenesis and not a myeloid progenitor cell as it was suggested by most authors.

P0475. *MYH*-associated polyposis with early-onset endometrial carcinoma and multiple pilomatricomas.

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It has recently been demonstrated that recessively inherited defects of the *MYH* gene, a DNA glycosylase involved in the base excision repair (BER) pathway, predispose to multiple colorectal adenomas and carcinomas through a significant increase of somatic G > T transversion in the *APC* and *KRAS* genes. In particular, the Y165C (exon 7) and G382D (exon 13) variants have been shown to play a pivotal role in Caucasian patients with *MYH*-associated polyposis (MAP). Herein we describe two unrelated Italian kindreds, both with consanguineous parents, characterized by unusual tumour associations. In the first kindred, the proposita presented with multiple adenomatous colonic polyps and an endometrioid endometrial carcinoma at the age of 45 years. She was found to be homozygote for the Y165C *MYH* mutation. In the second kindred, two affected siblings presented with multiple pilomatricomas and colorectal adenomas. A previously unreported *MYH* frameshift mutation, 1186-1187insGG (in exon 13), was present in the homozygous state in both siblings.
To our knowledge these are the first reports of an association between MAP and early-onset endometrial carcinoma and multiple pilomatricomas. These observations suggest that *MYH* mutations may predispose to additional tumours arising outside the gastrointestinal tract, possibly by increasing the rate of G > T transversions in specific target genes.

P0476. Array-CGH- and conventional CGH-analysis of laser-microdissected early breast cancer lesions - DCIS, LCIS and atypical hyperplasia

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A multistep model of breast carcinogenesis suggests a transition from normal epithelium to invasive carcinoma via non-atypical and atypical hyperplasia and carcinoma in situ. The introduction of mammographic screening has led to the increased detection of these preinvasive breast lesions and has caused problems for diagnostics and management of patients. Therefore, specific molecular markers are needed for objective classification and prognostication of early breast lesions.
The aim of our project is the identification and validation of new genetic markers in order to facilitate diagnostics of patients with early breast tumors. Four µm sections of paraffin-embedded breast lesions were dissected using laser capture microdissection (LCM). Additional sections were cut for interphase cytogenetics and immunohistochemistry analysis of p53, Ki67, Her2/neu, PR and ER. For CGH, whole genome amplification was performed by ligation-mediated PCR. Using this procedure, we analyzed 20 cases of ductal carcinoma in situ (DCIS), 15 cases of lobular carcinoma in situ (LCIS) and 10 cases of atypical ductal hyperplasia (ADH). None of these tissues showed any invasive cancer areas. Conventional CGH-analysis revealed frequent gains in DCIS on chromosomes 1q, 6p, 6q, 8q, 17q, 20p and 20q. Recurrent losses in DCIS were seen on 8p, 11p, 11q, 12p, 12q, 17p, 18p and 18q. Most cases of LCIS showed gains on 1q and a loss of the whole long arm of chromosome 16. The very same cases are now being subjected to high-resolution-array-CGH using our custom-built 6,000 BAC-clone array.

P0477. Detection of chromosomal imbalances in children's tumors

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The cytogenetic analyses of children's solid tumors are rare, due to low proliferative activity and difficulty in obtaining adequate number and quality of mitotic cells. In this study we present the results of cytogenetic analysis in 15 children with solid tumours. The aim of this study is to identify numerical and structural chromosome aberrations, and to determine the feasibility of using the interphase FISH method in the investigation of genomic imbalances. Cytogenetic investigations were performed on slides obtained by direct method of tumor tissue treatment. GTG- and CBG- banding method were used for chromosome identification. FISH analysis was carried out using locus specific and chromosome specific centromeric probes (Vysis). Analyzable metaphases were obtained from 14 specimens, and in one tumor the data on genomic imbalance were obtained by interphase FISH. Clonal chromosome abnormalities were found in 14 cases, while in one tumor normal diploid karyotype was identified. Most tumor presented numerical aberrations ranging from near-diploidy to near-octoploidy. Double-minute chromosomes were identified in three specimens, ring and telomere fusions in one. Aberrations of chromosome No. 1 were the most frequent clonal rearrangements. Interphase FISH revealed N-myc amplification in one out of three investigated samples and loss of the short arm of chromosome 17 in one patient. Our early results confirm that FISH is not suitable for initial screening of chromosomal aberrations in solid tumor, but is a valuable tool for detecting tumour-specific chromosomal aberrations that have diagnostic and prognostic significance.

P0478. Tissue microarray analysis of *erbB* oncogenes copy number alterations in squamous cell carcinoma of the larynx

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The copy number alterations of *erbB-1*(EGFR) and *erbB-2*(HER-2) have been investigated in a variety of solid tumors. To appreciate their role in the development and progression of squamous cell carcinoma of the larynx, we used probes for the 7p12 (EGFR) and 17q21 (HER-2) regions for fluorescence in situ hybridization.
A tissue microarray containing 1395 tumors was constructed and analyzed.
Amplification of *erbB-1* was found in 10.3% of the tumors in comparison to *erbB-2* - only 1%. The analysis revealed lack of correlation between the amplification of the oncogenes and the tumor phenotype as well as between the amplification of the oncogenes and the nodal involvement.
These results suggest that the copy number alteration of *erbB-1* and *erbB-2* are not significant for the tumor differentiation and progression.

P0479. Exon 20 of BRCA1 gene is involved in more than the half of mutation carriers identified in Greek patients with family history of breast ovarian cancer

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Hereditary breast and ovarian cancer syndromes can be caused by loss-of-function germline mutations in one of the tumor suppressor genes BRCA1 and BRCA2. In order to characterize these mutations in the Greek population we have been collecting samples from breast/ovarian cancer patients with family history in collaboration with Greek Hospitals. Our DNA bank contains samples from more than 350 patients, corresponding to approximately 300 families. In terms of family history this group consists of three subgroups (i) very early onset (<30yrs) without family history (10%) (ii) moderate family history (2 members affected, <50yrs) (40%) (iii) strong family history (3-7 members affected) (50%). Screening of *BRCA1* & *BRCA2* genes with direct sequencing, in 200 patients has revealed deleterious mutations in 43 unrelated patients. In exon 20 of BRCA1 we have identified three deleterious mutations in 26 patients, 12 carrying 5382insC, 3 R1751X and 11 carriers of G1738R. Other mutations identified in BRCA1 gene include a splice junction mutation in exon 23 (5586G>A) in two patients and six truncating mutations in exon 11 (1623del5, 3099delT, 3277insG, R1203X, 3741insA, 3896delT) in 6 patients. With respect to BRCA2 gene we have identified 7 different truncating mutations (in exons 2, 10 & 11) in 9 patients. Although there is an important number of novel or recurrent mutations identified with low frequency in both genes, approximately 60 % of the mutations are localized in exon 20 of BRCA1 gene. This fact facilitates the genetic screening in Greek patients with family history of breast ovarian cancer.

P0480. Birt-Hogg-Dubé syndrome in Dutch families

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Birt-Hogg-Dubé syndrome (BHD) is a rare autosomal dominant genodermatosis characterized by multiple benign skin tumours, designated as fibrofolliculomas. Affected individuals are at increased risk for pneumothorax, renal carcinoma and possibly other neoplasms. The *BHD* gene has been mapped to chromosome 17p11.2 and recently identified. The aim of our study was to identify and characterize Dutch families with BHD both at the clinical and molecular level. Patients were clinically evaluated and limited family data were collected. In addition, *BHD* germline mutation analysis was performed using DNA sequencing. At present 12 index patients with (suspected) BHD have been identified. Presumably, six cases are

sporadic and six familial. Neoplasms in index cases include breast and skin cancer. One family member died due to a mixed clear cell and papillary renal carcinoma. Pneumothorax was found in two cases. The first screening round for *BHD* germline mutations in three index patients revealed mutation c.1740dupC in two patients and c.875delC in one patient. Current findings indicate a large degree of clinical variability both between and within BHD families. Further insight into the tumour spectrum and genetic heterogeneity in BHD kindreds will direct counselling and preventive screening in the future.

P0481. Prognostic value of K-ras and p53 gene mutations in Turkish colorectal cancer patients

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Mutations of the p53 and K-ras genes has been reported to be of prognostic importance in colorectal cancers. In this study, K-ras and p53 gene mutations were examined for their prognostic value and their relationship with histopathological parameters of patients in stages I-II ($T_{1,2,3}N_0$) colorectal cancer. Tumor samples were collected from stages I-II colon-rectum cancer patients who underwent curative surgical treatment. No patient recieved any postoperative adjuvant therapy. Mutations in exons 5-8 of the p53 gene and codons 12 and/or 13 of the K-ras gene were assayed in paraffin-embedded tissue by PCR-SSCP method. A total of 43 cases (20 men; median age 64; range: 42-86) was collected. Twenty-eight (65%) patients had stage II cancer. Mutations of the p53 gene were found in 22 (51%) tumours. Six (14%) cases showed a mutation of the K-ras gene. In one case (2,3%) p53 and K-ras mutations coexisted. Five out of six K-ras and 12 of 22 p53 mutations were found in stage II cancer. The median follow up time was 76 months (range, 6-168). Fourteen patients developed recurrence, 11 of whom (79%) had tumors with K-ras or p53 mutation. The 5-year disease free survival rates indicated that no significant differences existed between the patients with wild type p53 (74%) or K-ras (71%) and mutant p53 (65%) or K-ras (0%). p53 mutations were found in high frequency in both stages (I and II) colon-rectum cancer but K-ras mutations showed high frequency only in stage II. None of these mutations showed a significant prognostic value.

P0482. p53 intron 3 16bp duplication polymorphism in Turkish women with breast cancer

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The functional and structural changes of p53 gene has a role in progression of breast cancer; the polymorphisms of p53 gene may affect phenotype of tumor. In this study, p53 16bp duplication polymorphism frequency and its risk factor for breast cancer were examined among Turkish population. Breast cancer samples (98 cases) were collected from paraffin embedded tissue. Controls (107) were collected from voluntear's blood who have no family cancer history and matched to case's age and sex. Touchdown-PCR and immunohistochemistry analysis were used. In our patients who have homozigot duplicated alleles (A2) were found %2, in controls %4, homozigots A1 alleles %60 in patients, %57 in controls and also heterozigots alleles were found %38 in patients, %39 in control. Since P53 16bp duplication polymorphism frequency was detected as %24 in white population, our result showed this polymorphism as %43 in Turkish population ($p < 0.001$). There is no significant correlation between the cases and the controls. Immunhistochemically mutant-p53-positive patients were found as %44. In the therapy of this desase, there is no correlation between the P53 expression and 16bp duplication polymorphism.. Although intron 3 16bp duplication polymorphism on p53 gene was observed with high frequency in Turkish population, this polymorphism was not a risk factor for breast cancer.

P0483. Detection of specific chromosomal aberration in de novo adult acute leukemia with FISH method

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We studied 23 patients over 18 years old with de novo acute leukemia. We carried out 26 FISH analyses with locus specific and centromere DNA probes (Vysis). In 13 of 26 analyses were found molecular changes.
Bcr/abl fusion gene was found in 3 of 7 analyses (range 30-95% of cells), were 1 case was with AML M5 and 2- with ALL;
MLL rearrangements were found in 3of 8 cases (range 70-100%)- 7 patients with M5 or M4 (MLL was presented in our study only in M5) and 1- with ALL and t(4;11). In 2 cases we proved cytogenetic findings with alteration of chromosome 11q23 and in 1 patient MLL was detected after unsuccessful cytogenetic analysis.
FISH for inv (16) was implemented in 3 cases with M4. Only in one of them was found inv (16) in 57% of the metaphases. Another 2 of 3 patients were with t (8; 21)(q22;q22)- rare genetic aberration in this FAB group.
The activation of C-MYC protooncogene was detected in 2 cases with L3 (Burkitt's leukemia) and t(8;14). In both of them was affected the central nervous system.
The investigation of -7/7q- was carried out in 2 patients with M6 and in 1 with M0. Clonal changes were found in all cases (range 44-64% of cells). These chromosomal aberrations were associated with bed answer to therapy and short remission.

P0484. Identification of People with an Increased Risk of Cancer

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Methods of genetic risk assessment include assessment of family history of disease and genetic testing. The aim of the present study was to identify and follow-up individuals at risk for cancer, and their families. Methods: Collecting family history information, using risk factors questionnaire and diet questionnaire. Knowing that women with first- and second-degree affected relatives have a significantly higher risk of developing breast cancer, that the risk increases with the number of affected relatives, younger age of onset, bilateral disease, and the occurrence of ovarian and breast cancer in the same relative within the affected lineage, we investigated the presence of these risk factors within families. Data collected from 300 women from investigated families are stored in order to initiate a cancer registry in the western part of Romania, to establish individuals at risk and the necessary preventing measures. Cancer genetic counseling often involves a multidisciplinary team of health professionals who have expertise in this area. The team may include a genetic counselor, genetic advanced practice nurse or medical geneticist, mental health professional, and medical expert such as oncologist, surgeon, or internist. Cancer genetic counseling may involve several family members, some of whom may have had cancer, and others who have not. In some cases, DNA-based testing can be used to confirm a specific mutation as the cause of the inherited risk, and to determine whether family members have inherited the mutation.

P0485. FISH analysis for diagnosis and disease management in myeloid leukemias

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Chronic Myeloid Leukemia (CML) and Acute Myeloid Leukemia (AML) are hematopoietic malignancies characterized by the proliferation of myeloid precursors. The hallmark of CML is the presence of Philadelphia (Ph^1) chromosome that results from balanced reciprocal translocation between chromosomes 9 and 22 leading to the formation of *bcr/abl* fusion gene. Similarly, the presence of certain cytogenetic anomalies marks different subtypes of AML, the most specific being t(15;17) in Acute Promyelocytic Leukemia (APML). The cytogenetic and molecular response in CML

patients (on IFN-α 2b therapy and after allogeneic bone marrow transplantation.) and in APML patients (on ATRA therapy and/or chemotherapy) was evaluated. Sequential cytogenetic analysis was done using standard methods in bone marrow samples from patients with myeloid leukemias. Further dual colour Fluorescence In Situ Hybridisation (FISH) analysis was done to assess molecular response using *bcr/abl* and *PML/RARα* probes in CML and APML patients respectively. The results of present study show that IFN-α and ATRA therapy induce both cytogenetic and molecular response in a significant proportion of CML and APML patients respectively thereby improving their prognosis and survival. The importance of using FISH analysis on interphase nuclei and poor-spread metaphases that cannot be analyzed using conventional cytogenetics was also highlighted. Further gene rearrangements could be identified in some CML and AML patients without any cytogenetic evidence of the translocation. Thus the present study stresses on the need for sequential cytogenetic and molecular analysis in diagnosis and disease management of myeloid leukemias.

P0486. Lack of 9q34 microdeletions in relapsed Ph-positive adult acute lymphoblastic leukemia patients

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In adult acute lymphoblastic leukemia (ALL) the t(9;22) has an incidence of up to 30% and is well described as the aberration with the worst prognosis. Microdeletions of 9p21 are frequently found in childhood ALL and are also described as an additional alteration in adult Ph-positive ALL. The target of these deletions is p16 (CDKN2A). Microdeletion of 9q34 as a secondary effect of the t(9;22) with the M-bcr breakpoint is frequent in CML and less frequent in adult Ph-positive ALL. In CML this deletion is associated with a poor prognosis and it has been suggested 9q34 microdeletions may worsen the unfavourable prognosis of Ph-positive ALL. Using FISH, we investigated 32 relapsed adult Ph-positive ALL patients for the presence of 9q34 and/or 9p21 microdeletions at primary diagnosis of the disease. Eleven patients (34,4%) showed deletions of 9p21. A hemicygous deletion 9q21 was found in ten patients; five patients had also a clone with a homocygous deletion of 9p21. In one patient combined hemicygous loss of 9p21 and 9q34 was due to a monosomy 9. No other patient with a microdeletion of 9q34 was found. We conclude, that 9q34 microdeletions do not contribute to the relaps risk in Ph-positive ALL.

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P0487. Evidence for the interaction of two gene loci (NQO1 and p53) regarding risk for spontaneous breast cancer

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Eight different SNP's in six different genes were investigated for possible association with breast cancer. We used a case-control study design in two Caucasian populations, one from Tyrol, Austria, and the other from Prague, Czech Republic. Two SNP's showed an association with breast cancer: R72P inTP53 and P187S in NQO1. Six SNP's, Q356R and P871L in BRCA1, N372H in BRCA2, C112R (E4) and R158C (E2) in ApoE and C825T in GNB3, did not show any sign of association. The P187S polymorphism in NQO1 was associated with breast cancer in both populations from Tyrol and Prague with a higher risk for carriers of the 187S allele. Combining the results of the two populations we observed a highly significant difference (p=0.0004) of genotype and allele frequencies (OR=1.46; 95% CI 1.16-1.85; p=0.001) and of the homozygote ratio (OR=3.8; 95% CI 1.73-8.34; p=0.0001). Combining the two "candidate" SNP's (P187S and R72P) revealed an increased risk for breast cancer of double heterozygotes (P187S/R72P) of the NQO1 and TP53 genes (OR=1.88; 95% CI 1.13-3.15; p=0.011) suggesting a possible interaction of these two loci.

P0488. Application of DHPLC in the molecular diagnostics of FAP

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Familial adenomatous polyposis (FAP) is inherited in autosomal dominant manner predisposition to develop numerous polyps in colon and rectum. FAP is caused by germ line mutations in APC gene. Described in 1991 the APC gene consists of 8,538 bp open reading frame and is predicted to encode 2,843 amino acids polypeptide. The APC gene mutations in most cases are small deletions or insertions. Single base substitution constitutes for 26-38% of the all detected mutations. Mutations screening is the first step of molecular diagnostics in carriers' recognition. Currently few methods can be applied for mutations screening and here we present denaturing high performance liquid chromatography for APC gene mutations analysis. 120 Polish FAP families were screened for mutations in coding APC gene sequence using DHPLC. In 33 FAP families 26 mutations types were detected and among them 17 mutations have not been described previously.

P0489. Implication of ETV6 and AML1 genes in the development of childhood B-lineage acute lymphoblastic leukaemia in Tunisia : molecular cytogenetic study.

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ETV6/AML1 fusion gene do to the translocation t(12;21) is seen in at least 20% of cases of childhood B-lineage acute lymphoblastic leukaemia. This translocation is, however, undetectable by classical cytogenetic study.
36 Tunisian cases of B-ALL, without detectable recurrent abnormality (i.e. translocations t(1;19), t(4;11), t(9;22) and hyperdiploidy), were studied by FISH using ETV6 and AML1 probes to identify the implication of these genes .
In 12 cases, karyotype from banding analysis showed a rearrangement of the 12p13 region. Among them, we detect in 2 cases ETV6/AML1 fusion do to the translocation t(12;21), in 1 case a fusion of ETV6 and MN1 do to the translocation t(12;22) and in 1 case a complex translocation t(5;11;12) with a fusion of ETV6 with an unknown partner located in 11q22. In 6 cases, we don't detect a fusion, but one copy of ETV6 gene was deleted.
In the other 24 cases, we detect the ETV6/AML1 fusion in 8 cases. We detect also, in 1 case, the implication of ETV6 gene in a translocation t(3;12)(q27;p13).
We conclude that, ETV6/AML1 fusion was implicated in 10 cases within 36 (27.8%).
ETV6 deletion was detected in 6 cases (16.7%), this deletion was, however detected in 50% of cases with cytogenetically detectable 12p13 rearrangement.In 2 cases, probable new partner genes of ETV6 were localised in the regions 3q27 and 11q22.

P0490. Complex variant Ph[1] translocations in chronic myeloid leukemia and acute lymphoblastic leukemia.

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Chronic myeloid leukemia (CML) is cytogenetically characterized by the finding of the reciprocal translocation t(9;22)(q34;q11), causing the formation of the Ph[1] chromosome. In 2-10% of the cases, the Ph[1] chromosome is generated by variant rearrangements, involving 9q34, 22q11, and one or more genomic regions.
We report seven cases of complex variant Ph[1] translocations diagnosed in six patients affected of LMC and one patient affected of acute lymphoblastic leukemia (ALL). Cytogenetic and FISH analysis with the use of WCP, CEP and LSI probes were carried out in bone marrow samples. The third chromosome involved in the seven complex variant translocations was the chromosome 1 (n=3), 5 (n=1), 11 (n=2), and 12 (n=1). FISH studies using the LSI BCR/

ABL.ES probe allowed the detection of the fusion gene bcr/abl on chromosome Ph[1] in all the cases, and loss of the ASS gene in two cases. WCP and CEP probes confirmed the complex translocations diagnosed by cytogenetics in 5 cases. In the remaining two cases a new cryptic reorganization was established. At diagnosis, all the chromosomally abnormal cells showed the complex variant t(9;22;V), suggesting that it was originated in stem cells. As far as we know, a new region, 12q13, found in one case, has not been previously described in complex variant translocations. All the breakpoints include genes already known to be related to neoplastic process.
This work has been partially supported by a grant (V-2003-Red-07-0) from the"Instituto de Salud Carlos III"

P0491. Absence of *ras*-gene hotspot mutations in canine fibrosarcomas and melanomas

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Point mutations within *ras* proto-oncogenes, particularly within the mutational hot-spot codons 12, 13, and 61, are frequently detected in human malignancies and in different types of experimentally induced tumours in animals. So far little is known about *ras* mutations in naturally occurring canine fibrosarcomas as well as *K-ras* mutations in canine melanomas. To elucidate if *ras* mutations exist in these naturally occurring tumours in dogs, in the present study we screened 13 canine fibrosarcomas, 2 feline fibrosarcomas and 11 canine melanomas for point mutations, particularly within the mutational hot-spots, making this the first study investigating a larger number of canine fibrosarcomas. None of the samples showed a *K-* or *N-ras* hot spot mutation. Thus, our data strongly suggest that *ras* mutations at the hot-spot loci are very rare and do not play a major role in the pathogenesis of the spontaneously occurring canine tumours investigated.

P0492. Evaluation of Telomerase mRNA (hTERT) in Childhood Acute Leukemia

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Human telomerase reverse transcriptase (hTERT) is the catalytic component of telomerase enzyme and has been shown to be associated with telomerase activity (TA). Although many studies in adult leukemia have been established to reveal the importance of TA, very few have been reported in the children. In this study hTERT levels in childhood leukemia was evaluated and compared with the prognostic factors described before.
The LightCycler instrument was used (online real-time PCR) for the quantification of hTERT in peripheral blood and bone marrow in 23 cases with acute lymphoblastic leukemia (ALL) and in 8 cases with acute myeloblastic leukemia (AML). Ten cases with normal peripheral blood and bone marrow were selected as control group. Cytogenetic analyses were available in 21 patients with leukemia.
In all cases with acute leukemia and in control group, peripheral blood hTERT levels correlated significantly with bone marrow hTERT levels. Before treatment, patients with ALL had significantly higher hTERT levels than that of AML patients and control cases. Among patients with ALL, higher hTERT levels were observed in patients with pre-B leukemia, followed by B cell and T cell leukemia patients. Initially increased hTERT levels decreased to the nearly normal levels during remission in cases with ALL. No correlation was observed between the initial hTERT levels and the known prognostic factors except cytogenetic findings. Higher hTERT levels were detected in patients having karyotypic abnormalities which indicate poor prognosis. hTERT levels might be useful in monitorization of the disease in leukemic children.

P0493. The association between mutator phenotype and the defects of MMR genes in AML and MDS patients.

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Recent advances in molecular genetic have allowed the analysis of many of the molecular mechanisms of cancer. One of these mechanisms is DNA repair, which controls the genetic stability of cells. Mismatch repair (MMR) gene defect is related to microsatellite instability (MSI) and it has been recognised as a common characteristic of several different types of human cancers. However, in acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS) there were few studies of the association between MSI and the defects of MMR genes.
In this study, the protein expression of three MMR genes (*hMSH2*, *hMLH1* and *hPMS2*) was examined by western blotting analysis in 35 patients (28 AML and 7 MDS) using three antibodies (anti-MSH2, anti-MLH1 and anti-PMS2). These patients were chosen from our previous published study. The mutator phenotype (RER+) was detected in 16 patients while 19 patients were in RER- group (sheikhha *et al*, 2002). Lack of expression of at least one protein was detected in 39.3% of the AML cases (11/28) and in 28.6% of the MDS cases (2/7). This rate was significantly higher (61.5%) in the RER+ AML patients (8/13) than the rate (20%) in the RER- AML patients (3/15). Lack of expression of hPMS2 protein alone was detected in only one patient. These results confirm that RER+ in AML and MDS is strongly associated with lack of expression of either hMLH1 or hMSH2 and, in few patients, hPMS2 proteins, whereas most of the RER- patients showed expression of all three proteins by western blotting analysis.

P0494. Reduced colorectal cancer risk in woman with SULT1A1 Arg213His variant

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Sulfotransferases are a superfamily of enzymes 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, particularly women,with His allele have a substantially lower enzyme activity. Several studies investigated the influence of this variant on susceptibility of colorectal cancer (CRC), but no definitive conclusion has been obtained.
We determined the allele frequency and genotype distributions of SULT1A1 Arg213His variant in 100 sporadic colorectal cancer patients and 146 normal controls from the Republic of Macedonia. The Arg/His and His/His genotypes were more common in controls than cases (64% vs. 50%, respectively, p=0.059, OR 1.74;95%CI 0.99-3.10), resulting in a His213 allele frequency of 40.0% in controls and 27.0% in cases (p=0.039). When we examined the data by age and gender, there was a statistically significant reduced risk of colon cancer only in women with the His213 genotypes and >60 years of age (OR 0.31;95%CI 0.12<O.R.<0.84, p= 0.0167) but not in woman of <60 year of age and in men (p>0.05 in all instances). No association was found in allelic frequency and genotype distribution of this variant when patients were grouped by other clinicopathological parameters (family history, localization, pTNM stage, MSI status). Our data indicate that the SULT1A1 Arg213His polymorphism has a protective role for the development of CRC in women >60 of age, most probably due to decreased biotransformation of procancerogenic compounds present in the diet in our population.

P0495. The canine *HMGA1*

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Due to the emerging advantages of numerous canine diseases as a genetic model for their human orthologs, the dog could join the mouse as the species of choice to unravel genetic mechanisms e.g. of cancer predisposition, development, and progression. However, precondition for such studies is the characterisation of the corresponding canine genes.
Human and murine HMGA1 non-histone proteins participate in a wide variety of cellular processes including regulation of inducible gene transcription, integration of retroviruses into chromosomes, and the induction of neoplastic transformation and promotion of metastatic progression of cancer cells.
Chromosomal aberrations affecting the human *HMGA1* gene at 6p21 were described in several tumours like pulmonary chondroid hamartomas, uterine leiomyomas, follicular thyroid adenomas, and others. Over-expression of the proteins of *HMGA1* is characteristic for various malignant tumours suggesting a relation between high titer of the protein and the neoplastic phenotype.In this study we characterised the molecular structure of the canine *HMGA1* cDNA, its splice variants and predicted proteins HMGA1a and HMGA1b. Furthermore, we compared the CDS of both splice variants for twelve different breeds, screened them for SNPs, and characterised a basic expression pattern.

P0496. Prostate Cancer and *CEACAM1* Genetic Variants: An Association Study

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Prostate cancer (PC) is the most commonly diagnosed malignancy among men in the western world. There is strong evidence that genetics plays an important role in cancer initiation and progression. Genes implicated in cell growth, proliferation and differentiation are important candidates in carcinogenesis. *CEACAM1* (carcinoembryonic antigen-related cell adhesion molecule 1) is a putative tumor-suppressor gene, located at 19q2, a region that has been linked with an aggressive phenotype of PC. Down regulation of CEACAM1 protein in cancer cells and its inhibitory role in tumor angiogenesis renders *CEACAM1* a plausible candidate for PC. We conducted a case-control association study of PC with six validated single nucleotide polymorphisms (SNPs) in *CEACAM1* from the NCBI database. The study sample consists of 577 histologically confirmed PC cases from 299 multiplex sibships and 576 unrelated controls, all of whom are of Caucasian ancestry. Of the studied SNPs, three located in exon 3 cause non-synonymous changes, two are intronic and one is located in the 3` untranslated region. One of the exonic SNPs, rs8111171, showed significant association (p = 0.032) with PC. Haplotypes constructed (Phase 2.0.2) with the three non-synonymous SNPs using one-sib case and the control group revealed a significant difference at one minor haplotype (p = 0.02). This is the first association study investigating *CEACAM1* in PC, and our results suggest that it has a possible role in prostate tumorigenesis.

P0497. Differential methylation of *LAMC3* and *TGFBR1* CpG islands in non-small cell lung and 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 MSRF we have identified four abnormally methylated CpG islands in DNA samples from breast cancer patients and ascribed two of them to 5' untranslated regions of laminin γ3 chain (*LAMC3*) and transforming growth factor beta receptor 1 (*TGFBR1*) genes. Laminin γ3 chain belongs to a recently characterized laminin 12 with detectable expression in most human tissues and organs including breast and lung. By methylation-sensitive PCR with specific primers we detected no methylation of *LAMC3* 5'-CpG island in control peripheral blood lymphocytes and apparently intact breast tissues. In breast and lung cancer samples methylation was shown in 13/40 (32,5%) and 5/40 (12,5%) respectively. Laminins are major glycoproteins of the basal laminae. In addition to an RGD sequence mediating cell adhesion, laminins contain motifs providing cell growth and differentiation via selective activation of signaling pathways dependent on protein kinase C. 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.
TGFBR1 take part in transforming growth factor β (TGF-β) signaling, mainly resulting in inhibition of cell proliferation. We have detected abnormal methylation of *TGFBR1* CpG island in 14/40 (35%) of breast cancer samples in the absence of methylation in control lymphocytes and breast tissue. *TGFBR1* null methylation was also found in 40 lung cancer samples. Characterization of *LAMC3* and *TGFBR1* expression in cancer samples as well as phenotype-genotype correlations are underway.

P0498. Determination of Her-2/neu gene amplification in breast carcinoma by fluorescent in situ hybridization (FISH)

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The study group was derived from the archive materials of 13,445 breast cancer patients examined by Prof. Dr. Hüsnü Göksel between 1960 and 2000. Preliminary results of the paraffin block samples of the first 48 cases to whom partial mastectomy was performed are presented here. In order to detect Her-2/neu status, FISH protocol was performed using Her-2/neu locus specific probe. Signals were counted and patients were classified in three groups according to signal numbers. Two signals were regarded as group I (n=31), 2-9 signals were regarded as group II (n=11) and 10 or more signals were regarded as group III (n=6).
Axillar metastasis ratios were 16%, 27% and 83% in groups I, II and III respectively (p=0.003). Axillary metastatic lymph node numbers and gross metastatic lymph node ratios were highest in group III (p=0.001 and p=0.01 respectively). Regarding local relapse and remote metastasis in 5 year follow up period, no significant difference was observed between the three groups.
Signal numbers decreased with ER expression (p=0.03). Histopathologically, irregular pattern of the tumor was observed in 100% of the patients in group III and 54% and 60% in groups I and II respectively (p=0.04). Hipercellularity of the tumor was significantly higher in group III (p=0.01). Extensive intraductal component ratio was significantly high in group III (p=0.04). The level of desmoplastic reaction and lymphocyte infiltration did not show significant difference between the groups.
In this ongoing study, preliminary results show that Her-2/neu signal numbers increase with worsening of clinical and histopathological malignancy criteria.

P0499. Matching patterns of genomic imbalances of hereditary and of non-hereditary retinoblastomas with biallelic RB1 inactivation point to common pathways of disease progression

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Genomic imbalances have been demostrated in 65-95% of Retinoblastomas (Rb) and are more frequent in Rb of older than in those of younger patients. Because patients with hereditary Rb are

younger than patients with non-hereditary Rb, we investigated if both groups differ from each other by the patterns copy number changes (CNC) as revealed by comparative genomic hybridization (CGH). Tumors of 60 patients with unilateral isolated non-hereditary Rb (nhRb) and of 61 patients with bilateral hereditary Rb (hRb), all with biallelic RB1 inactivation, were studied. Both tumor groups showed an increase of genomic imbalances with age which started at about 18 months. The fraction of abnormal tumors in patients <=18 and >18 months were nearly equal in hereditary and non-hereditary tumors (<=18 months: hRb 41.2%, nhRb 48.6%; >18 months: hRb 90%, nhRb 96%). There were no significant differences with respect to type and frequency of the chromosome regions involved in genomic imbalances between hRb and nhRb. The most frequent imbalances were gains of 6p (54.8% nhRb; 48.5% hRb) and 1q (42.9% nhRb; 27.3% hRb) and losses of 16q (38.1% nhRb; 21.2% hRb). We conclude that the matching patterns of genomic imbalances point to common pathways of disease progression in hereditary and non-hereditary retinoblastomas with biallelic RB1 inactivation.

P0500. Genetic Imbalances In Endometrial Hyperplasia And Carcinoma Detected By Comparative Genomic Hybridization

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To evaluate the sequential genetic events related to the development of precursor lesions and endometrial carcinoma, and its association with cellular atypia.
Paraffin embedded tissue specimens of 20 endometrial hyperplasia, 20 endometrial adenocarcinoma and 20 normal endometrial tissue were retrospectively evaluated by Comparative Genomic Hybridization technique. The average number of copy alterations (ANCA) index was used to define the incidence of genomic imbalances in each tissue group. Identified sequential genetic abnormalities were compared to the final histopathological diagnosis and the cellular atypia.
Seven hyperplasia and 14 carcinoma specimens had detectable and consistent chromosomal imbalances. The ANCA value was significantly correlated to the degree of cellular atypia and the grade of the tumors. Although 1p36-pter, 20q deletions and 4q overrepresentations were the most prevalent imbalances detected in both complex and complex atypical hyperplasias, 17q22-qter deletion and amplification of 2p34 were only seen in hyperplasias with atypical cells. Chromosomes 8q, 1q and 3q overrepresentations, the most frequent aberrations in endometrial carcinomas, were not detected in the precursor lesions. Chromosomes 1p36-pter and 10q underrepresentations were the other commonly seen aberrations in carcinomas, the latter being more frequent in well-differentiated lesions.
The pattern of chromosomal aberrations in precursor lesions were different from that observed in endometrial carcinomas, except the loss of 1p34-pter. Presence of 1p deletion in both endometrial hyperplasia and cancer specimens suggested that it is early event in development of carcinoma. These results supported a stepwise mode of tumorigenesis with the accumulation of a series of genetic aberrations in endometrial carcinogenesis.

P0501. Methylation of a number of tumor suppressor genes in various cancers.

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Aberrant methylation of normally unmethlated CpG-rich areas, also known as CpG islands, which are located in or near the promotor regions of many genes, has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancer. Thus, abnormal methylation serves as an alternative to the genetic loss of a tumor suppressor gene function by deletion or mutation.
We investigated the frequency of aberrant methylation of *p16*^{INK4a}, *p15*^{INK4b}, *p14*^{ARF}, *Rb1, HIC1, MGMT, N33, CDH1, ERα* and *CALCA* genes in different cancers, such as breast cancer (BC), non-small cell lung cancer (NSCL), retinoblastoma (RB) and acute leukemia (AL). The methylation status of investigated genes was determined by the method of restriction enzyme-related PCR (REP), methylation-specific PCR (MSP) and bisulfite genomic sequencing (BGS).
We determined that the investigated genes were differentially methylated in various cancers. *HIC1* and *CDH1* genes were more often methylated in BC, NSCL and RB samples, than in AL. Level of the *Rb1* gene methylation was approximately equal in all tumors samples. *MGMT, ER* and *N33* genes were low methylated in all tumors samples.
We also analysed a loss of *IGF2* gene imprinting in samples NSCL, BC, BR and AL. We investigated the demethylation of *IGF2* gene promoter by the REP. We determined that the loss of *IGF2* gene imprinting was characteristic of chosen tumors types.

	RB1	*p16/ CDKN2a*	*p15/ CDKN2b*	*p14/ARF*	*CDH1*	*HIC1*	*MGMT*	*N33*	*ER*	*CALCA*
BC	16% (14/85)	24% (20/85)	4% (3/85)	26% (22/85)	53% (45/85)	65% (55/85)	5% (4/85)	4% (3/85)	9% (8/85)	17% (15/85)
NSCL	20% (11/54)	43% (23/54)	1,8% (1/54)	35% (19/54)	74% (40/54)	81% (44/54)	0% (0/54)	15% (8/54)	7% (4/54)	20% (11/54)
AL	17% (14/90)	6% (5/90)	1% (1/90)	2% (2/90)	14% (14/90)	11% (10/90)	0% (0/90)	1% (1/90)	0% (0/90)	1% (1/90)
RB	25% (15/60)	15% (9/60)	0% (0/60)	22% (13/60)	60% (36/60)	47% (28/60)	2% (1/60)	0% (0/60)	7% (4/60)	3% (2/60)

P0502. Identification of chromosomal abnormalities using M-FISH in haematological malignancies

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Recently, novel molecular cytogenetic technique, termed multicolor fluorescence in situ hybridization (m-FISH), has been used to define complex chromosomal abnormalities, cryptic translocations and marker chromosomes in hematological malignancies and solid tumors.
We performed m-FISH analysis of ten bone marrow samples from three pediatric (ALL, AML, NHL) and 7 adult patients (4 AML, 2ALL, 1 CML).
In two AML and one CML patients, three specific complex translocation variants have been found as : t(1;7;22), t(8;16;21) and t(1;6;9;22). In other three cases, we have described the origins of the derivative chromosomes and complex karyotypes. And also we demonstrated three novel cryptic translocations for the first time in literature.
Our data suggest that cytogenetic results, which are important criterias in diagnosis, treatment and prognosis, should be appraised together with m-FISH, applied on the patient's undissolved karyotypes. So that more reliable results can be obtained for routine diagnosis and in research, m-FISH technique would help identification of localization of the novel candidate oncogenes.

P0503. Conventional and molecular cytogenetic findings of myelodysplastic syndrome patients

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Myelodysplastic syndrome (MDS) involves myeloid cells of the bone marrow which is important in progressive bone marrow insufficiency. The cells may transform to cause acute myeloid leukemia and these two diseases share clinical and cytogenetic features in common. 40 to 50% of all MDS patients have at least one chromosomal rearrangement. Loss of specific chromosomal regions like 5q- and 7q- are usually the secondary cytogenetic abnormalities associated with MDS.
In order to detect chromosome abnormalities associated with MDS,

bone marrow samples from 20 patients diagnosed as MDS were obtained prior to chemotherapy. Both conventional cytogenetic analyses and FISH methods were performed and locus specific probes for 5q, 7q, and inv(16) were used. Results obtained were compared. In one patient, the karyotype revealed del(20)(q11-qter), in another patient we observed 47 chromosome number with an extra marker chromosome, whereas another patient had del(5q15-qter) in 30% of his metaphases. Three patients with normal karyotypes revealed del(5q), two patients had del(7q) and two patients had inv(16). A total of 11 of 20 patients had chromosome changes visualized by either conventional or molecular cytogenetics (55%). Our results show that both methods are important in diagnosis and follow up of MDS patients, since they play different roles in detecting chromosome abnormalities.

P0504. Detection of BCR-ABL and PML-RARα transcripts by RT-PCR in Iranian Leukaemic patients

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The diagnosis of patients with CML may be carried out by a variety of techniques such as cytogenetic procedures, RT-PCR or FISH depending on disease status and course of treatments. The RT-PCR approach is most suitable in cases with Philadelphia chromosome negative results and minimal residual disease(MRD), monitoring progression or regression of the disease following treatments. RNA extraction and cDNA synthesis were performed according to the standard procedures. PCR amplification was carried out in either/and multiplex and nested formats for different possible translocations, and PCR products were electrophoresed and visualized on agarose gel. In this study 73 patients were examined for bcr-abl fusion gene by qualitative RT/PCR. Forty six of the patients were CML cases at different stages of the disease, and the rest were either ALL, AML or other cases. The mean age of the CML group was 45 years. Among our CML cases 7 patients were negative and 39 patients were positive for the fusion gene(84.7%): 20 belonged to major variant, 14 belonged to the minor variant and 5 cases were positive for both major and minor fusions. In the twenty five non-CML cases 12 patients were positive(48%): 8 for major and 4 for minor fusion genes. We also compared some RT-PCR findings with the cytogenetic results, among 38 cases 32(75%) had the same result. 6 were positive in RT-PCR but negative in cytogenetics. Three patients with AML-M3 phenotype were also examined for PML-RARα translocations. Two were positive for **s** form of this fusion gene.

P0505. Mutation screening of *SIAH1*, a candidate tumor suppressor gene in colorectal cancer

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About 3% of all colorectal cancer cases are hereditary and less than half of these can be related to the already known syndromes: FAP and HNPCC. The remaining families segregate as yet unknown disease causing genes. Patients in these families frequently have multiple adenomas and tumors are MSI negative.

SIAH1, the human homolog of the *Drosophila seven in absentia* gene, may be a candidate gene in these families. SIAH1 has been proposed to act as a downstream effector of p53 and overexpression of SIAH1 can mimic the effects of p53 activation and inhibit cell proliferation, promote apoptosis and suppress tumor formation. SIAH1 functions as a component of a complex which is proposed to target β-catenin for ubiquitin-mediated degradation in response to p53 activation. SIAH1 maps to chromosome 16q12-q13, a region frequently deleted in a large variety of tumors. All these observations suggest that SIAH1 may be a tumor suppressor gene. Eighteen families with hereditary colorectal cancer were included in a linkage study where eight families showed suggestive linkage, with a highest individual LOD score of 1.1, to chr 16q12-q13 (D16S3136 and D16S415). A mutation screening of the coding sequence of SIAH1 was performed in these families.

One sequence variant in codon 153, AAG to GAG causing an amino acid change from Lysine to Glutamine was found in all samples including the control samples, suggesting it to be a common polymorphism. Thus, in this study we did not find evidence for SIAH1 to be the disease causing gene in these families.

P0506. Prognostic Significance Of Cytogenetic Analysis In Children With Non-hodgkin Lymphoma

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Non-Hodgkin lymphoma (NHL) is a subgroup of malignant lymphomas caused by neoplastic proliferation of lymphatic cells. A significant number of patients with NHL have clones with aberrant karyotypes in the bone marrow. The most frequent chromosome rearrangements are: t(10;14)(q24;q11), t(11;14)(q13;q32), t(14;18)(q32;q21), del (11q), del (6q), del (7q), dic (9;12) (p13;p13), inv (2) (p23;q35).

In this retrospective study we present the results of cytogenetic analysis of 21 children with NHL, diagnosed at the Mother and Child Health Institute “Dr Vukan Cupic” in Belgrade, between January 1998 and December 2003. Cytogenetic analysis was performed on bone marrow cells, after 24h of cultivation and chromosomes were identified by standard banding techniques. Normal karyotype was found in 15 (71,4%) children. Chromosome abnormalities were seen in 6 (28,6%) children. Three patients had numerical chromosome aberrations: tetraploidy (92) in mosaic (2 cases) and near-diploidy (46±) in mosaic (1 case). In three patients structural chromosome abnormalities were found: deletion 5q33 in mosaic and complex structural rearrangments with t(1;14) and t(11;14) clones included (2 cases).

The results are compared with those reported in the literature.

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P0507. P53 Gene Mutations In Oral Squamous Cell Carcinoma

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Loss or mutation of the p53 gene is probably the most common single genetic change in cancer. p53 gene inactivation alters the protein structure, disabling its function as transcription factor in different pathways. Normal p53 protein arrests the cell cycle when DNA is damaged, until the damage is repaired. Tumour cells with mutant forms of p53 do not arrest in G1. Cells lacking normal p53 do not undergo apoptosis neither.

Recent studies show that one half of the cases of oral squamous cell carcinoma (OSCC) have mutations in the p53 gene. We have analyzed a large series of formalin fixed, paraffin-embedded samples of OSCC for the presence of the most common mutations in the p53 gene, distributed within exons 5-8. Mutation screening was done using PCR amplification followed by single-strand conformation polymorphism-SSCP. 38 out of 70 tumour samples harboured mutations (54%). The mutation distribution was as follows: 9 in exon 5 (13%), 8 in exon 6 (11%), 7 in exon 7 (10%) and 17 in exon 8 (24%) . Interestingly, three tumours had more than one mutation as shown by SSCP. There was no specific correlation between histologic grade or clinical stage and the presence of mutations. Namely, mutations were found in tumours of all grades and stages, with and without recurrences suggesting that p53 changes occur early in the development of OSCC. The mutations need to be confirmed by sequencing.

P0508. Epidermal growth factors receptor gene amplification in disseminated pediatric low grade gliomas

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Background: Disseminated low grade gliomas (LGGs) represent 5-10% of pediatric LGGs. The genetic and biological nature of these tumors is poorly understood. Epidermal growth factor receptor (EGFR) gene is located on the short arm of chromosome 7.

Amplification of the EGFR gene is a common finding in malignant gliomas. We looked for certain molecular abnormalities which may differentiate disseminated from the other LGGs.
Methods: Comparative genomic hybridization (CGH) was applied to 6 cases with disseminated LGGs and compared to 12 LGG controls. FISH analysis and immunohistochemistry were used to further highlight specific genetic targets.
Results: CGH revealed multiple chromosomal abnormalities in 5 of 6 disseminated cases and in 5 of 12 controls. There was a correlation between the amount of chromosomal abnormalities and clinical course. Amplification of chromosome 7 was noted in 4/6 cases as opposed to 2/12 controls (P=0.078). FISH analysis revealed EGFR amplification in two cases that were negative for amplification of chromosome 7 by CGH. Approximately 7.5% of the cells in these two cases exhibited high grade of amplification of the EGFR gene (more than 20 copies per cell) Immunohistochemistry for EGFR was positive in 6/6 cases and in 2/9 controls (P=0.08).
At a mean follow-up of 7.2 years all patients are alive with variable but slow disease progression.
Conclusions: High rate of EGFR amplification in disseminated pediatric LGGs may have implications on understanding of its role in gliomagenesis. Targeted therapies may be possible for these children. Larger scale studies are needed to further establish these findings.

P0509. A Report of Cytogenetic Study in 415 Iranian Leukaemic Patients

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This study was carried out on bone marrow and peripheral blood samples of 415 Iranian patients suspected of various types of leukemia between 1999 and 2003. The samples were referred from major hematology-oncology centers at Tehran. Cell culturing and banding (GTG & high resolution) were carried out according to the standard protocols. Chromosome analysis was performed following ISCN (1995) guidelines. The patients belonged to various leukemic categories, the major ones being: CML, AML, ALL, MDS and lymphoma. There were more male patients than females, approximately1.35:1 ratio. In terms of sample type, most cases had bone marrow aspiration whereas peripheral blood was utilized only in 44 cases. The common typical chromosomal abnormalities as well as rare and combined forms were observed. The overall chromosomal abnormality rate obtained was about 40%. The breakdown figures for different categories were roughly as follows: 65% in CML, 37.5% in AML, 28% in ALL, 22.2% in MDS and 19% in other types. Compared to published data, the observed chromosomal abnormality rate in the present study is considered low to average.

P0510. Prevalence of thrombophilic gene mutations in patients with oral cancer

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Growth and metastatic spread of tumors may be improved by utilization of the coagulation system. Nevertheless, the implication of thrombophilic gene mutations in cancer pathogenesis is still unclear. In order to investigate the possible contribution of the most common prothrombotic mutations in oral cancer, we studied blood DNA samples of 110 patients and 120 healthy controls of matched sex, age and ethnic origin (Greeks and Germans). For each patient, pathological diagnosis of tumor was based on biopsy examination, and a detailed family history of cancer or thrombophilia was collected. The prevalence of the factor V Leiden mutation, of the prothrombin G20210A mutation and of homozygosity for the methylenetetrahyd rofolate reductase (MTHFR) C677T substitution were analysed in the groups of patients and controls by PCR and restriction fragment length polymorphism methodology. The frequencies of most alleles and genotypes found were not statistically different among the two groups, or in compared subgroups in relation to sex, ethnic origin, age, age at onset, or type of oral cancer. However, there were significantly more MTHFR heterozygotes, and as a consequence, a significant increase in the C677T mutant alleles in the subgroup without family history of cancer ($p<0.05$). In addition, the subgroup of patients with a positive family history for thrombophilia had a significant increase of heterozygotes ($p<0.01$). Combined, these results may suggest that the MTHFR mutation is certainly not a major contributing factor in oncogenesis in the oral region, but may be still a minor one, in conjuction with decreased dietary uptake of folate.

P0511. Expression analysis of oncogens included in 11q13.5 amplicon co-amplified with the *MLL* gene in AML/MDS patients

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Amplification within chromosome arm 11q involving the mixed lineage leukemia gene (*MLL*) locus is a rare but recurrent aberration in acute myeloid leukemia and myelodysplastic syndrome (AML/MDS). Recently, we used microarray-CGH and FISH for characterization of amplified regions in a series of 13 AML/MDS patients with multiple copies of *MLL* gene. We have shown that in addition to the core *MLL* amplicon, independent sequences in 11q23-24 and/or 11q13.5 were co-amplified within the same cytogenetic markers. The first region, represented by clone bA90A13, was co-amplified in 83.3%, the second region represented by clone bA7H7 in 60% of AML/MDS cases. Both regions harbor a number of genes with possible oncogenic potential. In the present study we further delineate the amplified region in 11q13.5. The minimal amplified region of overlap around the core clone bA7H7 has been roughly estimated to 2.4 Mb, bordered by clones bA30J7 and bA153F6. Using semi-quantitative PCR we showed that in nine AML/MDS patients the minimal amplicon involves oncogenes GRB2-associated binding protein 2 (*GAB2*) and thyroid hormone responsive (*THRSP*), but not p21/Cdc42/Rac1-activated kinase 1 (*PAK1*). Results of a real-time RT-PCR based expression study of these genes in the patients with 11q13.5 amplification will be presented.

P0512. Lactase persistence and its relation to ovarian cancer risk in Poland

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The pathogenesis of ovarian cancer is not fully understood. Galactose, hydrolysing product of milk sugar lactose, has been hypothesized to be toxic to ovarian epithelial cells. Thus, consumption of dairy products and lactase persistence has been suggested to be a risk factor for development of ovarian cancer. Lactase gene expression and its activity is shown to be dependent on a variant C/T_{-13910} at the 5' end of the lactase (LCT, LPH) coding gene. In this study we determined lactase persistence/non-persistence status and its relation to Polish women with ovarian cancer.
The C/T_{-13910} variant was defined by solid phase minisequencing from 310 patients with ovarian cancer and 296 healthy controls.
The prevalence of lactose malabsorption was observed to be 33.1% among control samples versus 35.2% among ovarian cancer cases. Lactase persistence was not significantly associated with the ovarian cancer risk in Poland (OR = 0.91, CI 95% 0.65-1.28).
Based on our results lactase persistence does not increase the risk of ovarian cancer among women in Poland.

P0513. Gene expression study in neurospheres of glioblastoma origin and in differentiated neural cells using immunocytochemistry

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The aims of the study were : (1) to check the validity of the hypothesis according to which the glioblastomas are arising from brain stem

cells, the cells of glioblastoma biopsies were cultivated in the same medium as normal fetal brain neurospheres , and investigated the ability of these cells to form neurospheres and differentiate into the neurons and glial cells . And (2) to compare the expression of some transcription and other factors in neurosphere and differentiated from them cells. We were able relatively easy to isolate from glioblastoma biopsies (in 8 of 10 cases) the cells growing like neurospheres, and clone them; and show that both the original neurosphere cells and their clones can be differentiated into the cells expressing neuronal and glial markers. The comparison of the expression patterns of different factors in the cells of neurospheres , and in the differentiated from them cells shows that this system could be well used in the studies of neural differentiation in malignant gliomas. And having the knowledge that both the neurospheres and cloned cells are able to differentiate into the cells expressing neuronal and glial markers, and taking into consideration the great cellular heteromorphism found by us both in neurospheres and cloned cells, we propose that it could be truly quite possible, that the glioblastomas are arising from the brain (genetically changed) stem cells. The study is supported by Estonian SF grant nr. 5250 and grant TARMPO421.

P0514. A Report of Cytogenetic Study in 415 Iranian Leukaemic Patients

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This study was carried out on bone marrow and peripheral blood samples of 415 Iranian patients suspected of various types of leukemia between 1999 and 2003. The samples were referred from major hematology-oncology centers at Tehran. Cell culturing and banding (GTG & high resolution) were carried out according to the standard protocols. Chromosome analysis was performed following ISCN (1995) guidelines. The patients belonged to various leukemic categories, the major ones being: CML, AML, ALL, MDS and lymphoma. There were more male patients than females, approximately1.35:1 ratio. In terms of sample type, most cases had bone marrow aspiration whereas peripheral blood was utilized only in 44 cases. The common typical chromosomal abnormalities as well as rare and combined forms were observed. The overall chromosomal abnormality rate obtained was about 40%. The breakdown figures for different categories were roughly as follows: 65% in CML, 37.5% in AML, 28% in ALL, 22.2% in MDS and 19% in other types. Compared to published data, the observed chromosomal abnormality rate in the present study is considered low to average.

P0515. *hMLH1* G224D variant is pathogenic in HNPCC

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Hereditary nonpolyposis colorectal cancer (HNPCC) is frequently caused by MMR gene mutations. Most *hMLH1* missense mutations are unlikely to be act as major causative factors in HNPCC (Genuardi et al, 1999). The *hMLH1* G224D variant in exon 9 causing an amino-acid change has been reported previously in HNPCC (Pensotty et al, 1997). While the variant has been shown to impair protein function (Scherbakova et al, 1999; Trojan et al, 2002) it remains as unclassified variant at the ICG-HNPCC registry. We present a Spanish pedigree that fulfils the Amsterdam criteria for HNPCC carrying a G244D (GGT to GAT) variant at the *hMLH1* gene.
27 relatives were available for study. Seven of them have been diagnosed of HNPCC-related tumours (colorectal, endometrial or glioblastoma) (mean age: 49.8; range: 32-63). All affected patients carried the G244D variant and corresponding tumors showed microsatelitte instability. In two of these cases loss of MLH1 protein has been detected. At risk versus not at risk analysis: Four of the 20 non-affected relatives were non-symptomatic carriers (mean age: 43.4; range: 29 - 57). The remaining 16 did not carry it (mean age: 47; range: 20 - 83, 4 of them been older than 60 years). In conclusion, our results support that G244D should be considered as a pathogenic mutation in HNPCC.

P0516. Retinoblastoma gene expression is frequently altered in chronic lymphocytic leukemia

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B cell chronic lymphocytic leukemia (B-CLL) is the most frequent form of leukemia in Western countries. The pathogeny of B-CLL involves both deregulated proliferation and inhibition of cell death. A particular role in the regulation of these phenomena is played by proteins involved in early G1 phase regulation among other by pRb gene.
We have investigated cell cycle genes expression (pRb, p16) on the level of its mRNAs and proteins in non-stimulated and stimulated cultured B-CLL lymphocytes. For estimations of mRNA we have used RN-ase Protection Assay (h-CC2 multiprobe template set), the protein level was studied by Western blotting and immunocytochemically.
We observed variable level of pRb mRNA in 24 studied B-CLL patients. 10 out of 24 patients were characterized by undetectable or very low level of pRb expression (8 patients with now expression of pRb) or by medium/high transcriptional activity of that gene. pRb protein had nuclear localization. After stimulation pRb mRNA level increased significantly only in 50% of the studied B-CLL but remained unaltered in the rest of cultured B-CLL. The level of pRb was in reverse correlation to expression of p16 gene.
Given the essential role of pRb gene in cell cycle we have concluded that differences in pRb gene expression among patients suffering from B-CLL may contribute to heterogeneity of that neoplasia and might have an impact on its progression. Intrinsic or regulatory abnormalities of the RB gene may be responsible for no response in expression level after stimulation by mitogens in part of B-CLL patients.

P0517. Mutational Load Distribution Analysis. A Novel Non-Invasive Tool For Pancreatic Cancer Risk Assessment

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The sequential analysis of the mutational spectra at loci involved in the pathogenesis of pancreatic tumors may reflect cumulative pancreatic cancer risk. We have assessed whether Mutational Load Distribution Analysis (MLDA) of pancreatic juice may reflect the risk of pancreatic cancer before the tumors emerges. Patients and methods. Series 1. Five cases with no evidence of pancreatic pathology or malignancy after ERCP for biliary disease; 5 chronic pancreatitis and 5 carcinomas. Series 2. 8 chronic pancreatitis and 16 pancreatic carcinomas. Series 3. Sixteen samples from 8 members of 3 families carrying a p16 germ line mutation. DNA was extracted from pancreatic juice obtained during ERCP. An RCA enhanced zip-code array that enables the simultaneous detection of 22 mutations at K-ras and p53 genes was used. Results: MLDA separated the three groups of series 1 based on the total number of mutant alleles (mutational load) and on the level of the highest allele (distribution). In controls no single allele constituted more than 1.2% of the molecules examined. An allele constituting more than 3.8% indicated the presence of carcinoma. In chronic pancreatitis -at risk category- the frequency of the predominant allele was 1.2-3.8%. Results of series 2 validated the initial findings. MLDA in p16 mutation carriers showed an at risk pattern with fluctuating values upon time. In conclusion, MLDA of pancreatic fluids yields can be an effective tool for the longitudinal assessment of pancreatic cancer risk.

P0518. Cytogenetic findings in natural killer-cell lymphoma/leukemia.

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Natural killer (NK)-cell lymphoma/leukemia is a group of rare but

highly aggressive neoplasms. They occur predominantly in the nose/nasopharynx, sometimes in extranasal sites, or rarely as a leukemic form, and are thus categorized into: extranodal NK/T cell lymphoma nasal type, and aggresive NK cell leukemia. NK-cells are characterised by expression of CD2, cytoplasmic CD3ε and CD56, and germline T-cell receptor (TCR) genes. The great majority of cases harbour Epstein-Barr virus (EBV) in a clonal episomal form.
A variety of cytogenetic abnormalities has been described, but so far no specific chromosomal rearrangement has been identified. In most of cases, the chromosomal changes involve loss or gain of genetic materials, the most frequently described are del(6)(q21-q25) and i(7q). Recently, structural rearrangements in 8p22-23 and 12p13 have been also described. We report here the clinical features and cytogenetic findings in four cases of (NK)-cell lymphoma/leukemia.
Conventional cytogenetics and FISH studies were carried out in bone marrow and/or peripheral blood samples. In three cases an abnormal karyotype was detected, standing out two structural chromosomal rearrangements with 12p13 and 8p22 breakpoints, and none 6q or 7q abnormalities. The involvement of the genes, located in the breakpoints, in the pathogenesis of NK-cell lymphoma/leukemia will be discussed.
This work has been partially supported by a grant (V-2003-Red-07-0) from the “Instituto de Salud Carlos III”

P0519. Mutation analysis in breast cancer patients from Northwest Germany: A report from Muenster as part of the national Consortium for Hereditary Breast and Ovarian Cancer

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Inherited mutations in the genes BRCA1 and BRCA2 present a lifelong risk for the development of breast and ovarian cancer. Therefore comprehensive identification of gene lesions should represent state of the art diagnostics. Consequentially in 1996, in Muenster, Germany, a centre for counselling, therapy, psychological care and genetic testing was founded in the framework of a nation wide network supported by the Deutsche Krebshilfe.
Despite huge efforts using up-to-date technology e.g. DHPLC or complete sequencing, a recent review summarizing data obtained all over Germany showed that in less than one third of all patients deleterious mutations could be identified (Muenster 22%). Because the vast majority did not show any unambiguous pathogenic mutation, these patients are either linked to still unknown cancer predisposing genes or current technology is insufficient to identify all disease causing mutations in these genes.
To evaluate the proportion of gross genomic rearrangements in the BRCA1 gene, we therefore analysed 50 breast cancer patients using the recently developed multiplex ligation dependent probe amplification technique (MLPA; MRC Holland) and identified a duplication of exon 13 and a deletion of exon 17 within the BRCA1 gene. Interestingly two cases of exon 18 deletion turned out as false positive due to heterozygous sequence variations in the ligation site (c.5214Y and c.5215R).
These preliminary data suggest that although rearrangements within the BRCA1 gene seem not to be frequent in the German population MLPA turned out to be a sensitive, cost-effective and rapid method for the detection of genomic rearrangements.

P0520. Identification of relevant genomic aberrations in renal cell carcinoma by array-based comparative genomic hybridization and complementary expression profiling

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Renal cell carcinoma (RCC) accounts for 3% of all malignancies. The clinical manifestation of RCC is highly variable, many patients are affected by metastatic disease. The prognosis of metastatic RCC remains dismal as no effective treatment exists. Tumor biology is partly related to the histopathological subtypes of RCC. The most common subtype, clear cell RCC (ccRCC), is strongly associated with somatic deletions of chromosome 3p. Other common chromosomal alterations in progressing ccRCC include deletions of 6q, 8p, 9, and 14q, and duplication of 5q. Although some of these genomic aberrations appear to be linked to clinical outcome, specific genes have not yet been identified.
In order to understand the underlying molecular mechanisms causing different biological behaviour we performed a parallel analysis of 19 ccRCC specimens and patient-matched normal tissue by array-based comparative genomic hybridization and expression profiling. The genomic microarray contained 2300 elements allowing to detect copy number changes across the genome with an average resolution of 1.5 Mbp. Expression profiling was carried out using microarrays of 3600 nonredundant sequence-validated human cDNAs selected on their putative involvement in tumorigenesis. Differentially expressed genes were confirmed by reverse quantitative PCR.
Classification of tumors based on their genomic and expression profile was achieved by cluster analysis. Profiles were used to stratify tumors according to clinical behaviour. Congruent changes in genomic loci and gene expression were correlated and are the basis for further functional analysis. Candidate ‘progression suppressor’ genes will be discussed.

P0521. Investigation of a region on chromosome 17 in non-BRCA1/2 Swedish breast cancer families.

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Breast cancer is the most common malignancy in women of the Western world. A large proportion of familial breast cancers are caused by known mutations in the breast cancer genes BRCA1 and BRCA2. However the frequency of mutations in these genes varies greatly between populations and there still remains a significant fraction of breast cancer families without attributable mutations in BRCA1/2. Breast cancer families in the Stockholm region of Sweden exhibit a very low frequency (<10%) of mutations in either of these two genes and as such it is believed that unknown novel breast cancer genes are responsible for disease within this population.
A combined approach utilising comparative genomic hybridisation (CGH) with previous loss of heterozygosity and linkage analysis data was used in an attempt to localise novel regions harbouring predisposing genes.
CGH analysis revealed loss of either chromosome 17 or chromosome 6 in high risk breast cancer families. Chromosome 17 was further investigated as these results were in agreement with previous LOH data and available genotyping data. Further fine mapping of a possible region shared by all families, indicated the smallest possible region of interest to lie between markers D17S1880 and D17S1293. Genes located within this region were screened for germline mutations using both dHPLC and direct sequencing.
No truncating mutations were identified, 3 silent polymorphisms were identified in 2 genes and shown to be carried by all affected family members.

P0522. The role of hMLH3 in a subset of Swedish colorectal cancer families

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Colorectal cancer is one of the leading causes of cancer death in Western countries. Several syndromes, such as FAP and HNPCC, are known to be associated with increased colorectal cancer risk, and disease causing genes in these syndromes have been identified. However these syndromes can be found in only small proportion of colorectal cancer families (2-3%).
To map genetic loci that harbor unknown colorectal cancer predisposing genes, we are carrying out a genome-wide linkage analysis using 18 colorectal cancer families from Sweden. All families were previously tested negative for mutations in the APC, MLH1, MSH2 and MSH6 genes. Linkage is assessed by multipoint parametric linkage analysis. Five families showed suggestive linkage for a region on chromosome 14q between markers D14S276 and D14S74, which harbors the hMLH3 gene. Mutations in the hMLH3 gene are involved in a small proportion of atypical HNPCC families. Considering the fact that evidence of linkage was seen in families with same phenotype (atypical HNPCC), hMLH3 is a good candidate. To determine the role of hMLH3 gene in families exhibiting linkage to chromosome 14, we have performed mutation screening of this

gene. Both, genomic and cDNAs are tested using DHPLC, direct sequencing and RT-PCR. Results will be presented.

P0523. Metabolic and DNA repair genetic polymorphisms and survival from bladder cancer in men.

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In industrialized countries bladder cancer is the fifth most common cancer in men. The detoxification “pathways” and the removal or repair of DNA damage have a key role in protecting the genome of the cell from the insults of cancer-causing agents. Polymorphisms in metabolic and DNA repair genes have been identified and extensively studied in relation to the modulation of cancer risk, but few studies have been conducted on the relationship between genetic polymorphisms and survival from cancer.
In the context of a survival study on 322 incident cases of transitional-cell bladder cancer, we investigated the survival status in relation to 13 polymorphisms in three metabolic genes (GSTM1 P/N, GSTT1 P/N, NAT2 R/S, 137 cases) and six DNA repair genes (XRCC1-28152A/G, XRCC1-26651A/G, XRCC1-26304T/C, XPD-35931A/C, XRCC2-31479G/A, XRCC3-18067C/T, XRCC3-17893A/G, XRCC3-4541C/T, ERCC4-30147A/G, PCNA-6084G/C, 231 cases). All subjects are men aged 40-74 followed up for mortality for an average of 48 months (range 1-100, median 54 months, SD 30.7): of them, 66 died and 256 were still surviving at the end of follow-up. Mutations in p53 were determined in bladder tissue of 45 subjects and resulted associated with shorter survival (p=0.03).
None of the metabolic or DNA repair polymorphisms showed a statistically significant relationship with survival but ERCC4-30147A/G, whose variant allele is nevertheless under-represented in the study cohort and needs confirmation on a larger sample.
Further studies on genetic polymorphisms and bladder cancer are warranted since metabolic and DNA repair polymorphism might influence survival by modulating the response to drugs thus allowing individualized therapies.

P0524. Detection of T439I mutation of BRAF in sinonasal malignant melanoma

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Oncogenic activation of the BRAF, a critical serine/threonine kinase in the RAS/mitogen-activated protein kinase pathway, has been demonstrated to be involved in the pathogenesis of cutaneous malignant melanoma (CMM). The pathogenetic mechanism is frequently based on activating somatic mutations, for instance a V599E amino acid substitution that enhance the kinase activity, which has been described in >60% of CMM and premalignant melanocytic lesions. The V599E mutation is by far the most common mutation found in CMMs. Activating mutations of BRAF have been described in exon 11 and 15. Whereas a lack of BRAF mutations was found in uveal malignant melanomas (UMM). Data on mucosa associated malignant melanoma (MMM) is rare, it seems that the frequency of BRAF mutations is lower than in CMMs. We describe a T439I mutation detected in a MMM, a sinonasal melanoma of a 72 year old female patient. The mutation involves the second amino acid of exon 11 of BRAF. This amino acid represents a known inhibitory phosphorylation side of BRAF. The activating effect of the loss of this phosphorylation side could be demonstrated in vitro. To our knowledge this is the first detection of this mutation in melanoma.

P0525. The *BRCA1* Exon 13 Duplication is present in the Swedish population

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Germline mutations in the *BRCA1* gene predispose to breast and/or ovarian cancer. Although most of the known mutations to date are point mutations or small deletions and insertions, an increasing number of large chromosomal rearrangements has been reported in the last years. A duplication of a 6-kb fragment including exon 13, ins6kbEx13, is considered to be a common founder mutation originating from Northern Britain.
Here, we report the finding of this exon 13 duplication in two apparently unrelated multiple case breast-ovarian cancer families from Northern Sweden. Both families had been screened negative for *BRCA1* and *2* germline mutations by direct sequencing and PTT. Linkage analysis showed that both families were linked to the *BRCA1* locus and shared a common haplotype. In order to test for large chromosomal rearrangements, multiplex ligation-dependent probe amplification (MLPA) was carried out. The results indicated a gain of genetic material for *BRCA1* exon 13. A PCR-based test for the ins6kbEx13 mutation was performed and confirmed the presence of this mutation in our samples.
To evaluate the frequency of this duplication in Swedish breast cancer families, we tested patients from 46 breast-ovarian and 142 breast cancer only families. The mutation was identified in one of the 188 families (0.53%). We therefore conclude that this mutation is rare in the Swedish population; it should however be considered in the diagnostic procedure in cases where phenotype and family history strongly suggest a mutation in the *BRCA1* gene, but no mutation can be detected by standard screening methods.

P0526. Clinical genetic analysis of neuroblastoma patients from Serbia and Montenegro

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Neuroblastoma (NB) is one of the most frequent malignant childhood solid tumor. Recent genetic and biological studies have led to better understanding of this disease. In the present study 47 patients with NB were diagnosed at the Mother and Child Health Institute of Serbia “Dr Vukan Cupic”,between January 1997. and June 2003. Among 47 NB patients 12 children (25,53 %) were under the age of 1 year.Cytogenetic analysis was performed on 34 (72,34 %) patients.Normal karyotype was found in 22 patients patients (64,7%). Abnormal karyotype was found in 12 patients (35,29 %): 2 cases of near diploidy(±46) in mosaic (both at stage IV),2 cases of near- triploidy (±69) in mosaic (one at stage II and one at stage IV),4 cases of near-tetraploidy (±92) in mosaic (all at stage IV),one case of tetraploidy (92)(at stage IV),1 case with homogeneously staining regions (HSRs) and double minute chromosomes (DMs)(at stage III),one case with deletion 1p36 (at stage IV), and one case with complex karyotype (at stage IV).Chromosome 1p36 analysis was performed on twenty three patients using FISH (19 cases) and PCR (4 cases).MYCN analyses were performed on 27 tumors by FISH.Seven of 27 NB tumors (25,92 %)(3 at stage III, 3 at stage IV and one at stage IVs) were with amplified MYCN.
The authors will discusse the prognostic value of genetic results taking into account potential confounding factors such as stage and age.
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P0527. Correlation between the results of genetic scrennning for BRCA1/2 mutations and immunostainning for the BRCA1/2 proteins.

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Due to the complexity of BRCA1/2 genetic screening, it would be useful to have additional methods to better select patients in families with uncertain risk and to decide which gene to screen first. Objective:to study the correlation between the immunostaining

of BRCA1/2 proteins and the results of the genetic screening of BRCA1/2 mutations.Methods: 1)BRCA1/2 mutation screening: After genetic counselling, 6 high risk patients consented in genetic screening. Aliquots of whole genomic DNA extrated from peripheral blood were amplified by PCR, using primers that covered the whole exonic sequence of BRCA1. PCR produts were analized in a CSGE gel and positive samples sequenced automatically. Immunostainning: parafine blocks of breast and/or ovarian tumors from the same patients were analized using specific monoclonal antibodies for BRCA1 and BRCA2 proteins. The reaction was either negative if no nuclear distinctive labeling was observed (damage of the protein) or positive if nuclear labeling observed (integrity of the protein). Results: 2 patients with BRCA1 mutations had immunostaing of their tumors negative for BRCA1 and 3 patients who are BRCA1 negative had immunostainning positive for BRCA1. The 6th patient had an indeterminate result for BRCA1 screening and immunochemistry showed normal function of BRCA1 but was negative for BRCA2. BRCA2 screening for this patient is underway. Conclusion: These results suggest a good correlation between the two methods. We will extend this analysis to other patients screened and to BRCA2 mutations as well. BRCA1/2 immunochemistry may contribute to the individuals belonging to hereditary breast/ovarian cancer families.

P0528. Frameshift mutations in exon 1 of the RB1 gene can be associated with incomplete penetrance of retinoblastoma

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Most RB1 gene mutations result in premature termination codons or loss of regions that code for functionally essential domains of pRB. Carriers that are heterozygous for mutations of this kind almost invariably develop retinoblastoma. Incomplete penetrance of retinoblastoma is seen in some rare families only. These show distinct mutations including (i) promoter region mutations resulting in deregulated levels of normal protein, (ii) small in-frame alterations resulting in only partial loss of normal function, and (iii) mutations in introns that possibly result in leaky aberrant splicing.
In the course of routine genetic testing, causative RB1 gene mutations were identified in 594 index patients (337 with bilateral/familial retinoblastoma, 227 with isolated unilateral retinoblastoma). Excluding unaffected parents with mutational mosaicism, incomplete penetrance of retinoblastoma was found in relatives of 22 patients. Mutations in 13 of these families can readily be assigned to one of the functional classes outlined above. However, 4 families with incomplete penetrance showed frame-shift mutations in exon 1 (g.2078insC, g.2113del26, g.2126del38, g.2131del7). In these families there were a total of 8 mutation carriers without retinoblastoma, two patients with unilateral retinoblastoma, and 4 patients with bilateral tumors. RNA from peripheral blood was available from affected and unaffected mutation carriers from one family. RT-PCR showed that the mutant transcript is expressed in family members with and without retinoblastoma. These results show that genetic testing is important in parents of patients with frame-shift mutations in exon1 because, contrary to expectations, mutations of this kind can be associated with incomplete penetrance.

P0529. Telomere length and telomerase activity and expression in acute leukemias in children

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The objective of this study was to see whether there is any relationship between telomerase activity and expression and telomere length in leukemic cells. In addition we would like to investigate the possible alternations in telomerase activity and expression and telomere length in children with acute leukemias at the moment of diagnosis and during remission. High telomerase expression and activity has been found in cells of ALL and ANLL as well as in myeloblast cell line K562. Only in some cases of peripheral blood lymphocytes from healthy individuals low telomerase expression and activity has been detected. Chemiluminescent detection of terminal restriction fragments (TRF) from DNA isolated from ALL cells showed variable pattern of telomere length. The ALL cell appeared to have both long and short telomere lengths, in contrast to normal lymphocytes producing limited pattern of TRF (short telomeres).The ANLL cells produced predominantly short telomere pattern despite high telomerase activity and expression. Telomerase activity was diminished in patients in remission during chemotherapy. Telomere length in ALL cells was shorter in remission as compared to the time of diagnosis. It can be concluded that high telomerase activity and expression in leukemic cells do not correlate with telomere length (TRF pattern).
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P0530. X Chromosome Inactivation and Breast Cancer

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Skewed X-chromosome inactivation (XCI) may be equivalent to a functional loss of heterozygosity for putative X-linked tumor suppressor gene(s), and may represent a new mechanism for development of hereditary cancers based on the studies in young breast and invasive ovarian cancer patients (Kristiansen et al. *J. Med. Genet.* **39**, 30-33, 2002; Buller et al. *J. Natl. Cancer Inst.* **91**, 339-346, 1999). However, the correlation between BRCA status and XCI in breast cancer was not studied. We investigated the XCI status of 248 female breast cancer patients and 220 controls by use of a polymorphic CAG repeat in the androgen-receptor gene. Blood DNA samples were amplified by PCR directly and after digestion with the methylation-sensitive restriction enzyme *Hpa*II and densitometric analysis of the alleles performed. Skewed X-inactivation (>80% skewing) was observed in 80 of the 190 informative patients (42.1%), and 10 of the 124 informative controls (8.1%) (P<0.001). >90% inactivation of one allele (extreme skewing) was present in 42 (22.1%) patients, and in only three controls (P<0.001). Among BRCA mutation carriers, skewing was observed in three of 16 *BRCA1* (18.8%), and four of six *BRCA2* (66.7%) carriers. In the group with familial clustering without BRCA mutation, skewing was observed in seven of 15 (46.7%) patients. Lastly, 66 of 149 (44.3%) sporadic cases were skewed in their XCI patterns. Our results show a statistically significant association between the occurrence of skewed XCI and prevalence of breast cancer among *BRCA2*(+), familial and sporadic (P<0.001), but not in *BRCA1*(+) patients (P=0.116).

P0531. On detection of radiosensitivity in breast cancer patients by the GoMNT

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The micronucleus test (MNT) is frequently used for biomonitoring environmental exposure to mutagens or to assess DNA repair capacity after a mutagen challenge in G0 at the start of the lymphocyte cultures. This G0 MNT has been used to demonstrate a higher radiosensitivity in a proportion of breast cancer patients. This proportion varied greatly between different studies. When we carried out a large (91 patients, 96 controls) own study we observed a high percentage of radiosensitive patients (more than 70%) with the difference between patients and controls varying greatly

between different observers (from OR = 1.2 p = 0.8 to OR = 17.7 p < 0.0001). Lymphocyte cultures were irradiated with 2 Gy at the start, treated with Cytochalasin A for the induction of binucleated cells during the last 24h, and slides prepared as usual. The slides were coded, stained with Giemsa and the micronuclei were counted in at least 500 binucleated cells according to international guidelines. It was not surprising that the ability of the observers to discriminate patients and controls had a strong correlation with their cytogenetic experience (from 3 months to 5 years). Repeat counting by the same observer demonstrated, however, a very good reproducibility of the counts (Coefficient of Variation between 0.055 and 0.095). This observation indicates that the power to detect differences in the number of induced micronuclei between patients and controls depends on criteria which are not defined with sufficient precision in the international guidelines.

P0532. Mixed germ cell-sex cord tumors associated with gonadal dysgenesis in young patients

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Gonadoblastoma is an unusual mixed germ cell-sex cord stromal tumor that occurs mostly in individuals with abnormal gonadal development and karyotype with Y-chromosome or Y-derived sequences. Half of the gonadoblastomas show germ cells overgrowth leading to a typical dysgerminoma transformation. The age at diagnosis is variable but they are very rare at early ages. We present 5 patients with different types of gonadal dysgenesis, in 4 of them (cases1-4) the tumor was recognized in early childhood while in case 5 a disgerminoma was observed at age 15. In these patients we found different types of tumors: juvenile granulosa cell tumor beside a gonadoblastoma, bilateral gonadoblastoma and disgerminoma. FISH analyses performed to evaluate the X and Y cell distribution between the tumor and the disgenetic gonadal tissue, showed that Y cells were more frequent in the tumor than in the gonad. We discuss these findings and the genesis of these tumors in young children and recommend early surgical management of the dysgenetic gonads

P0533. INK4 familiy genes - Expression in human acute leukemia cells

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INK4 family genes are members of cyclin dependent kinases inhibitors(CDI) -class of proteins which inhibit cyclin-dependent kinases (CDK) activity during cell-cycle(especially G1 phase) and that way control progression through the cell-cycle. INK4 family consists of four genes p16, p15, p18, p19. Their mutation are rare but their incorrect expression is observed in many cancer and hematological malignancies and often is connected with poor prognosis. The alteration of these genes were also found in acute leukemia, but there are still limited range of studies to establish importance of these results for prognosis and treatment.
In our research we examined the level of mRNA INK4 family genes in 34 bone marrow samples obtained from patients with acute leukemia before treatment. In the examined group 19 patients have acute myeloid leukemia(AML) and 15 - acute lymphoblastic leukemia (ALL). For analysis mRNA expression we used semiquantitative method: Multi Probe RNase Protection Assay System(RiboQuant). We also correlated our results with clinical data.
In majority of examined leukemic cells we observed low level of p16 and p15 mRNA, only 2 LLA samples and 3 AML samples show high level of p16 mRNA. P19 mRNA was very low or absent in 68% of AML samples and 74% of LLA, and p18 in 47% of AML samples and 60% LLA, but in 3 MLA samples p18 mRNA level was significantly higher than in other samples.. The comparison of our results with different clinical data give us possibility to make more different observations, which need further research.

P0534. Could Mycoplasma-Mediated Oncogenesis be Responsible for the Formation of Conventional Renal Cell Carcinoma

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The aim of this study is to investigate the association between the conventional Renal Cell Carcinoma (cRCC) and Mycoplasma sp. infection. Normal (N), Renal Intratubular Neoplasia (RIN) and tumor (T) tissue samples from 33 patients with cRCC and 35 healthy controls were studied. Nested PCR was performed in two steps with seven primers (4 outer, 3 inner) which can recognize at least 15 different Mycoplasma sp. DNA was analyzed in the 3% agarose gel. Mycoplasma sp. DNA was detected in 36%, 67%, 82% of N, RIN, T samples respectively. No Mycoplasma sp. was detected in none of three tissue samples from 6 out of 33 cRCC patients. Mycoplasma sp. DNA was detected only in 5 out of 35 (14%) cases of the samples taken from the control group.
It is the first time that an association between RCC and Mycoplasma has been investigated. Significantly a higher existence of Mycoplasma sp. DNA was detected in tissues of cRCC patients compared to that of healthy control group. It can be deduced that mycoplasma-mediated carcinogenesis may have a role in the development of cRCC. We propose a novel pathway to explain the multi-stage oncogenesis mechanism which may be responsible for the formation of cRCC.

P0535. Cytogenetic findings in pediatric renal cell carcinoma

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Adenocarcinomas of the kidney are rare childhood tumors and present less than 0,1% of the malignant tumors in children. Less than 20 cases with chromosomal abnormalities have been reported and neither their karyotypic characteristics nor the molecular mechanisms behind pathogenesis are clear. We have cytogenetically analysed short-term cultured cells from a highly differentiated renal adenocarcinoma in a-8-year-old girl. The girl was brought to the surgical emergency room upon mild abdominal trauma. She complained immediately on heavy left ventral abdominal pain and was in pre shock on admission. Ultrasound disclosed a 10 cm left abdominal tumor with suspected haemorrhage. CAT-scan examination confirmed the finding. Preoperative treatment with i.v. Vincristine and Actinomycin-D was instituted according to the SIOP-9301 protocol for Stage III presumed (ruptured) Wilms' tumor. Left nephrectomy was performed four weeks later. Following uneventful surgery, external beam therapy was given with 15 Gy, 10 fractions, targeting the tumor bed. No further therapy was given and the girl is completely well almost 5 years later. A near-triploid karyotype with only numerical aberrations was found in 5 out of 17 analysed metaphases: 58-60,XX,-X,-1,+7,-8,-9,-11,-14,-15,+17,-18,-19,-21,-22 [cp5]/46,XX[12]. Histopathologic examination showed an adenocarcinoma.
Most common rearrangements in pediatric renal cell carcinomas are translocations involving chromosome X. Only one case with sole numerical abnormalities has previously been published. Both the present and published case showed gains of chromosomes 7 and 17. It has been implied that numerical changes as sole anomalies in malignant tumors could be consistent with a better prognosis.

P0536. Genetic counselling and surveillance strategies in individuals at high tumor risk in pancreatic cancer families

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Pancreatic cancer (PC) is an aggressive disease with a poor prognosis. It is estimated that 5-10% of PC cases are familial. An 18-53-fold increased risk has been reported in first degree relatives of PC patients in pancreatic cancer families (FPC). Germline

mutations of the genes BRCA2 or CDKN2A are considered to confer an up to 10- or 22-fold increased risk for PC development in mutation carriers, respectively. Up to date, 50 families have been enrolled in the German national case collection for FPC (FaPaCA) after detailed genetic counselling. BRCA2 and CDKN2A mutation screening was performed after written informed consent. A total of 90 high risk individuals has been identified among 273 family members including 19 individuals with BRCA2 or CDKN2A germline mutations. Results of the mutation screening are disclosed to individuals in an interdisciplinary counselling session with a human geneticist, a surgeon and a psychooncologist. All high risk individuals are offered a screening program for early PC detection. Female BRCA2 mutations carriers are advised to participate in specialised breast/ovarian cancer screening and/or prophylaxis programs. CDKN2A mutation carriers are told to adhere to surveillance for early melanoma detection. The ongoing study will help to assess the risk for tumor development in FPC families with or without germline BCRA2/ CDKN2A mutations prospectively, and to identify additional genetic markers prediposing for PC.
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P0537. Electronic microarray-based KRAS2 mutation detection to evidence colonic tumor DNA in sera.

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Among recent technological advances, DNA chip devices, which allow automated, high-throughput genotyping, promise to considerably improve the detection capability of mutations in clinically relevant samples. We took advantage of the Nanochip® Molecular Biology Workstation (Nanogen™) and the recently introduced microelectronic array technology, to develop a method suitable to detect KRAS2 mutations in circulating DNA. Mutated KRAS2 genes were the first tumor-specific gene sequences detected in the blood from patients with cancer. KRAS2 mutations are found frequently in several types of commonly occurring human cancers, especially cancer of the pancreas, colorectum, lung, and thyroid. When mutated KRAS2 sequences are detected in blood, they seem to be associated quite specifically with cancer. Moreover, KRAS2 gene mutation is usually at codon 12, and rarely at codon 13, 25 or 61: so the detection assays can be focused on these positions. After a classical quantitative PCR to evaluate the circulating DNA concentration, we used the electronic chip technology to first assess the presence of KRAS2 gene mutation and second to evaluate the mutated/normal DNA ratio in serum. Mutations in circulating non-cell-associated DNA have been detected in the plasma and serum of colon cancer patients. It is postulated that this DNA is released directly into the circulation from tumor cells undergoing apoptosis, we showed here, that not all this DNA in serum derived directly from the tumor but also from normal cells. Finally, the electronic microarray technology provides the opportunity to easily detect DNA from colonic tumor in patient serum.

P0538. Allelic loss on Chromosome 17p13.1, at p53 region, and Microsatellite instability (MSI) make clear a diatribe on the origin of a „second lesion“ in a child with Medulloblastoma

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Diagnosis of Medulloblastoma was made in a 5-year old child with severe neurological impairment, by MR. Radio-chemio and a second successive chemio treatments were carried out during few weks. Six months after the surgery removal of the primary lesion, the child showed again severe, neurological, symptoms. New MR evaluation revealed new meningeal involvement, interpreted as post-radiotherapie damage. Rachidocentesis didn't reveal the presence of neoplastic cells.
We decided to perform the molecular analysis of DNA extracted from the cells smear on slides , and we obtained important results: we found LOH of two loci (VNTR at the intron 1 of p53 gene, and D17S945 from the critical region of p53 gene), identical to the LOH revealed in the primary lesion represented by Medulloblastoma. It was possible to well interpretate the clinical manifestations as progression of the primary lesion, and exclude the damage by radiotherapie. The pathology examination confirmed the results of the molecular clonality analysis.

P0539. Development of a Rapid Detection Assay for Carcinoma and Adenocarcinomas Patients Using Green Fluorescent Single-chain Fv Specific for Carcinoembryonic Antigen (CEA)

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Recombinant antibody fragments and their conjugates have great potential in the field of tumor detection and therapy, and bypass major limitations related to the applications of conventional monoclonal antibodies such as poor tumor targeting, inadequate tumor penetration and immunogenicity. To develop effective immunoreagents for diagnostic and therapeutic applications, highly specific tumor markers are required. CEA is a well-established tumor-associated antigen, highly expressed in colorectal carcinoma and frequently elevated in adenocarcinomas of the lung, breast and other gasterointestinal organs. Moreover, the sugar-chain structure of CEA produced by cancer cells is different from those of the normal counterparts of CEA family. From a phagemid library of mouse scFv, several high affinity CEA-specific binders were obtained after four rounds of panning. Chimeric molecules were then developed containing the fluorescent properties of enhanced green fluorescent protein (EGFP) and the antigen binding properties of the selected scFvs. These recombinant molecules were able to recognize cell lines as well as tissue samples from cancer patients expressing CEA. These fluorescent immunoreagents are very effective for diagnostics, and they can complement or even bypass immunological and immuohistological methodologies in cancer detection.

P0540. Evaluation of FAK gene expression in cancer cell lines using Quantitative Real Time RT-PCR

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Focal adhesion kinase (FAK) is a cytoplasmatic tyrosine kinase, expressed in most tissues examined so far. FAK is over expressed in tumors and most of the evidence suggests that overexpression is a marker for invasive and metastatic tumors. Consequently, a role has been suggested for FAK in the development of invasive cancers. The origin of this overexpression is unknown. Increased dosage of the FAK gene may contribute to the elevated protein expression but overexpression for this protein may have postgenomic origins.
Using molecular techniques (RT-PCR and Real Time Quantitative RT-PCR) the postgenomic level (transcription) has been investigated in seven tumoral cell lines (two derived from solid tumors and five from hematological malignancies and lymphomas), in the Department of Medical Cytogenetics CHU Clermont-Ferrand.
Extraction and isolation of total RNA for all tumoral cell lines have been made according to the laboratory procedures. RT-PCR and Quantitative RT-PCR (Light Cycler System -Roche Molecular Biochemicals) have been developed according to a standard protocol two tube- two step, using the primers for FAK gene (references Genebank NM 005607) and for GAPDH gene (control and standard curves, Reference Genebank M17851). Analysis of amplification products by RT-PCR showed a band of 147 bp for FAK and 433 bp for GAPDH. No detectable RT-PCR product was observed for two lines: HL-60 and U937. High level copy number for cDNA corresponding to FAK gene was observed in K562 cell line, intermediate level for KG1a, A549 and M4Beu and low level for Jurkat cell line.

P0541. Analysis of Tumor Necrosis Factor-a (TNFA) and Lymphotoxin-a (LTA) polymorphisms in children with cystic fibrosis and chronic airway disease.

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Cystic fibrosis (CF) is an autosomal recessive lethal disease characterized by progressive obstructive lung disease and pancreatic dysfunction. The severity of airway disease in CF modified by secondary genetic factors. Chronic airway disease (CAD) is the common disease of childhood after asthma.
Inflammation of the airway wall is a principal feature of the airway disease and it may therefore be of interest to identify factors that could directly influence the intensity of airway inflammation. TNF is an inflammatory cytokine that found in increased concentrations in chronic respiratory patients airways. Probably, polymorphisms in the TNF and LTA genes are in relation to airway inflammatory diseases.
In order to investigate the possible roles of gene polymorphisms in the development and progression of CF and CAD we genotyped 62 patients with CF, 181 children with severe non asthmatic CAD and 216 control subjects for both TNFA and LTA polymorphisms.
The frequency of GG genotype of TNFA gene was significantly higher in patients with CAD (81.8%) compared to control (65.1%) ($\chi2=12.53$, $p<0.001$; OR= 2.39).
The rare LTA GG genotype was found in 10.2% of CF subjects ($\chi2=4.42$, $p<0.03$;
OR= 3.96).
We observed association of CF with TNFA-LTA (GG-AG) combination (36.6% versus 22.4% in control; OR= 2.00 CI 1.00-3.91).
Significant association were found between CAD and TNFA-LTA (GG-AG and GG-AA) combinations (79.0% versus 65.0% in control; OR= 2.06 CI 1.20-3.37)
Our results suggest that TNFA and LTA levels may be important in the pathogenesis of severe lung disease at CF and CAD patients.

P0542. Rapid Genetic Screening Assays for Alpha1-Antitrypsin Deficiency

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Alpha1-antitrypsin (AAT) deficiency is one of the most common lethal genetic diseases worldwide. It leads to jaundice in infants, liver disease in children and adults, and pulmonary emphysema in adults. Mutations in the PI gene, located on chromosome 14, are associated with AAT deficiency. The most common risk alleles are PiS (1/9-1/12 carrier frequency in Caucasians) and PiZ (1/30 to 1/40 in Caucasians).
We have developed two simple and rapid detection methods for the PiZ and PiS mutations, based on a single nucleotide primer extension reaction, followed by ELISA in a kit format. The ProntoPlex™ AAT assay consists of a multiplex primer extension reaction of the Z or S allele in a high throughput screening format. Both mutations are tested simultaneously in one well, while another well tests for the normal allele in the Z mutation site. This format allows the identification of homozygotes for the PiZ allele, which is the most common deficiency variant. Positive samples can be verified using a full genotyping assay on the Pronto® AAT Verification Strip, where each mutation is tested in two wells (*mut* and *wt*). The combination of a general screening kit with the Verification Strip™ provides a complete and inexpensive tool to find AAT mutation carriers.
Pronto® AAT, the alternative kit, is a full genotyping assay, in which each mutation is tested in two wells. This format has the added benefit of a positive internal control for each tested sample.
Molecular diagnosis of AAT deficiency would allow timely and effective treatment.

P0543. Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo

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Amylin (also designated islet amyloid polypeptide; IAPP) is a member of the calcitonin family of hormones that is co-secreted with insulin by the pancreatic beta-cells. Cell culture assays have suggested that amylin could affect bone formation and bone resorption, this latter function following its binding to the calcitonin receptor (CTR). The present study shows that Amylin-deficient mice display an osteoporosis-like phenotype (approximately 50 % bone mass reduction) that is due to an increase in bone resorption while bone formation is unaffected. In vitro, amylin is shown to inhibit fusion of mononucleated osteoclast precursors into multinucleated osteoclasts in an ERK1/2-dependent manner. While Amylin +/- mice displayed a low bone mass phenotype and increased bone resorption parameters, Ctr +/- mice display a high bone mass due to an increase in bone formation parameters. Moreover, compound heterozygote mice for Ctr and Amylin inactivation displayed bone abnormalities observed in both Ctr +/- and Amylin +/- mice thus ruling out that amylin uses CTR as its main receptor to inhibit osteoclastogenesis in vivo. Thus amylin is a physiological regulator of bone resorption that acts through an unidentified receptor and whose deficiency may contribute to the osteopenia of type I diabetes patients.

P0544. New CARD15 mutation in an Italian family affected with Blau Syndrome

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Blau syndrome (BS) is a rare chronic granulomatous syndrome characterised by arthritis, uveitis, skin rash, captodactyly and autosomal dominant inheritance. Its responsible gene has been mapped to the 16q21.1 region and recently identified as the CARD15 gene, encoding a bacterial product receptor involved in natural immunity which is also involved in susceptibility to Crohn disease (CD).
While its CD-associated CARD15 variants (R702, G908R, L1007fs) affect the C-terminal Leucine Rich Repeat Domain, and result in loss of the NF-kB activating function, BS has been associated with missense changes in the Nucleotide Binding Domain (NBD); R334Q/W and L469F which are not found in healthy subjects and result in increased NF-kB activation.
In the only known Italian BS family, followed for many years in Padova we identified by Direct sequencing in both affected mother and daughter a new mutation in the NBD, a heterozygous G1147A nucleotide substitution predicted to cause E383K. DHPLC analysis of exon 4 encoding the NBD (AA 273-577), using the Wave system (Transgenomic), excluded its presence in seven healthy relatives and 100 controls. The affected subjects do not carry other mutation known to be associated with BS or CD, nor any variant of probable pathogenic significance.
The E383K mutation affects a highly conserved Glu residue which is changes into a positively charged Lys. This change could influence the function of the Walker B motif as a Mg2+-binding site. Further functional and structural studies should be done to fully understand the pathogenisity of this new mutation.

P0545. X-inactivation patterns in female Beckwith-Wiedemann patients

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Beckwith Wiedemann syndrome (BWS) is a congenital overgrowth condition caused by several multigenetic factors. BWS occurs at a much higher frequency than is expected in Monozygotic (MZ) twins, with these twins most often being discordant for the BWS phenotype. Indeed, a recent study reports the incidence of BWS among both male and female MZ twins as being 8%, compared with a general population incidence of 0.3-0.4%. In addition, female BWS MZ twins made up 6.4% of the reported 8% MZ twinning frequency. These findings are also in accordance with numerous other studies. The

high proportion of BWS discordant MZ twins has led researchers to believe that the process of X-inactivation may be involved in this discordance. In this study we focus on the X-inactivation pattern among singleton females with varying multigenetic causes of the BWS phenotype. We also investigate the parental origin of the X chromosomes in subjects displaying a skewed X-inactivation pattern. The X-inactivation pattern among all subjects was investigated by analysis of the highly polymorphic (CAG)n repeat of the androgen receptor (AR) gene. The enzyme *Hpa II* was used to specifically digest gemonic DNA containing the nonmethlyated (CAG)n repeat and the resulting polymerase chain reaction (PCR) yielded only the inactive, methylated X chromosome. Results will be shown.

P0546. A novel mutation in FGD1 gene (Aarskog syndrome)

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Faciogenital dysplasia (FGDY), also known as Aarskog-Scott syndrome, is an X-linked developmental disorder characterized by disproportionately short stature, macrocephaly and by facial, genital and skeletal anomalies. The FGD1 gene, responsible for faciogenital dysplasia, was mapped to the Xp11.21 region. It is composed of 18 exons that span over 51kb of genomic DNA. The FGD1 cDNA encodes a 961 amino acid protein that acts as a guanine nucleotide exchange factor (GEF) and specifically activates the p21 GTPase Cdc42, a member of the Rho family of GTPase proteins. Rho GTPases play a critical role in the regulation of the actin cytoskeleton in a wide variety of eukaryotic cells.
To date only very few mutations (3 point mutations and a deletion of 3 exons) in the FGD1 gene have been described in affected families. We now report the finding of a novel FGD1 mutation, a stop mutation in exon 2 (E128X) in a sporadic case from Germany.

P0547. New aspects of the moelcular basis of tuberous sclerosis

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Tuberous sclerosis is an autosomal dominant tumor suppressor gene syndrome. The genes TSC1, encoding hamartin, and TSC2, encoding tuberin, are responsible for this disease. TSC genes have been shown to affect cell size control. Our group has shown that TSC genes function as potent cell cycle regulators at the transition from the G1 phase to S phase by affecting the stability of the cyclin dependent kinase inhibitor p27 (JBC, 1997, 272:29301; PNAS, 1998, 95: 15653; Hum. Mol. Genet., 2000, 9:1721; Oncogene, 2001, 20:4904; Oncogene, 2003, 22: 4786). Until now, the molecular mechanism how TSC genes affect p27 stability remained elusive. We now found that tuberin directly binds to p27 in vivo. This interaction regulates p27 activity via affecting its SKP2-mediated proteolysis, its localization and its binding to 14-3-3 proteins. This is the first demonstration of the direct interaction of a tumor suppressor involved in a genetic disease with the cyclin dependent kinase inhibitor p27.

P0548. LGMD2A in Bulgarian patients caused by the 550delA CAPN3 gene mutation

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Limb-girdle muscular dystrophy type 2A (LGMD2A) is caused by mutations in the muscle specific calcium-activated neutral protease 3 (CAPN3) gene, resulting in a calpain 3 protein. Most of the detected mutations (single nucleotide substitutions, small deletions/insertions) are private and scattered along the whole gene. Considering that the 550delA mutaion in exon 4 was reported as very frequent in patients from Russia, Ukraina and Turkey, we expected that this mutation would be also found in Bulgarian patents.
We used SSCP method to detect the 550delA mutation.
We analyzed 33 patients clinically diagnosed as LGMD of unknown type and LGMD2A was confirmed in 6 patients (18%): 4 were homozygous and 2 were heterozygous for the 550delA mutation.
We screened 58 DMD/BMD sporadic cases without mutation in the dystrophin gene and 550delA mutation was found in 4 cases (7%): 2 homozygous patients, 1 heterozygous and in 1 case the 550delA mutation was combined with large deletion including the whole exon 4 of the gene.
We screened 51 SMA type III patients with genetically unclear diagnose and LGMD2A was confirmed in 2 cases (4%): 1 heterozygous patient and 1 with 550delA mutation combined with large deletion.
Our results demonstrate that the 550delA mutation in the CAPN3 gene seems to be very frequent in the Bulgarian LGMD2A patients, too.
LGMD2A could be also considered in doubtful and genetically not confirmed DMD/BMD and SMA III cases because of the common clinical findings in these neuromuscular disorders.
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P0549. Mutation and polymorphism analysis in the human EPM2B gene in Lafora disease patients

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Progressive myoclonus epilepsy of the Lafora type or Lafora disease is an autosomal recessive disease characterized by epilepsy, myoclonus, dementia and Periodic acid-Schiff-positive intracellular inclusion bodies. Two genes have been described as responsible of Lafora disease: EPM2A and EPM2B. EMP2A, located on chromosome 6q24, encodes for a putative tyrosine phosphatase called Laforin. EPM2B (denoted as NHLRC1) is located on 6p22 and encodes for a putative E3 ubiquitin ligase called Malin. We now report haplotype and mutational analysis of the EPM2B gene in 32 chromosomes from 16 affected individuals. Eight novel mutations were found: two nonsense mutations (W219stop and E67stop), one 6bp microdeletion (c880-885del6), one 4bp insertion (c1017insATCT) and four missense mutations (C68Y, E67Q, P264H and D233A). To date a total of 13 mutations in EPM2B have been found in our family set, including previously reported mutations. We also have characterized two simple nucleotide polymorphisms (332C-T and 18C-G). Haplotypes were constructed in order to determine haplotypic associations between EPM2B polymorphisms and EMP2B mutations. P69A is the predominant mutation and was found in 10 chromosomes. The variability of the haplotypes associated with the P69A mutation supports the idea that P69A is a recurrent mutation with several phylogenetics origins. All other mutations were found only in one or two chromosomes. We conclude that a remarkable genetic allelic heterogeneity is present in Lafora disease associated to mutations in EPM2B, and even the most common EPM2B mutations have several phylogenetic origins. In our study, 73% of patients presented mutations in EPM2A and 27% in EPM2B.

P0550. Transciptional profiling in SURF1 deficient Leigh Syndrome

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Leigh syndrome (LS) is a phenotypic manifestation of a variety of disorders of energy metabolism, most commonly COX deficiency. Because of locus heterogeneity associated with the clinical phenotype, identifying the molecular basis for this disease and other mitochondrial disorders remains difficult. We are interested in designing alternative diagnostic strategies such as microarray analysis. Total RNA samples extracted from the cultured human fibroblasts were assayed by microarray analysis using the 18K Affymetrix chip. Data analysis was performed using both public domain (dChip) and commercial software (GeneSpring and Rosetta). We found that a candidate gene for LS, the COX assembly factor Surf1, was down-regulated approximately 4 fold, whereas other known assembly factors such as SCO2, Cu Chaperone, COX11, COX10 and COX15 were unchanged. The sequence analysis to the Surf1 gene of this patient revealed one 10bp truncation followed by a 2bp insertion within exon 4 on one allele, and a mutation from Q196 to stop codon on the other allele, resulting in two null alleles.

Gene Set Enrichment Analysis (GSEA) showed that other genes involved in the OXPHOS pathway were up-regulated, including UCP-2. Furthermore, the expression of genes involved in energy metabolism down stream of the OXPHOS pathway was also altered. For example, HIF was up-regulated 4.7 fold, and iNOS was up-regulated 2.7 fold, while VEGFB was down-regulated approximately 5 times. Thus, transcriptional profiling potentially offers an alternative approach, additional to the established diagnostic tests for clinical cases where molecular basis of disease exhibits genetic heterogeneity.

P0551. Chimeric *CYP21P/CYP21* and *TNXA/TNXB* genes in the RCCX module

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In the RCCX module in chromosome 6p21.3 two types of chimeric gene are described, *CYP21P*/*CYP21* and *TNXA*/*TNXB*. To date, four chimeric *CYP21P*/*CYP21* genes have been found as the defective *CYP21* genes in congenital adrenal hyperplasia (CAH). These molecules are composed of a 5' *CYP21P*-specific sequence in common, but differ in the 3'- *CYP21*-specific sequence. Among them, only one retained minor enzyme activity of the 21-hydroxylase. The other three chimeras had no functional activity. As a result, the organization of the gene array is -*C4A*-*CYP21P*/*CYP21*-*TNXB*- in the RCCX module. However, the chimeric *TNXA/TNXB* gene is caused by *CYP21* deletion leading to a partial *TNXB* replaced by *TNXA* sequence to produce the array -*C4A*-*CYP21P*-*TNXA*/*TNXB*- . From recent studies, two such molecules have been found. The two structures differed in the interchange region of the *TNXA*/*TNXB* hybrid, which in one case was located between IVS 44 and exon 44 of the *TNXB* gene and in the other extended to 120-bp beyond exon 36 of the *TNXB gene*. Both chimeric *CYP21P/CYP21* and chimeric *TNXA/TNXB* produce a 3.2-kb fragment on *Taq*I digestion. Therefore, we conclude that these two chimeric genes are two distinct molecules and the chimera *TNXA/TNXB* is involved in recessive Ehlers-Danlos syndrome as well as 21-hydroxylase deficiency.

P0552. Alpha-globin gene deletion and point mutation analysis among in Iranian patients with microcytic hypochromic anemia.

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ABSTRACT:

Alpha-thalassemia (α-thal) is one of the most common single-gene diseases in the world. It is caused by a variety of deletional and non-deletional α-globin mutations, leading to a reduction or complete absence of gene expression. The genetic icidence for this disease varies between 1% and 98% throughout the tropics and subtropics. In this study we have tested 235 Iranian individuals, randomly chosen from a pool of patients with low MCV, low MCH, normal or slightly reduced Hb levels and normal HbA_2. Two single-gene deletions (-$\alpha^{3.7}$, -$\alpha^{4.2}$), five double gene deletions (--^{SEA}, --^{MED}, --^{THAI}, --^{FIL}, -$(\alpha)^{20.5}$), and five point mutations (Hb Constant Spring, Hb Quong Sze, Hb Pakse, Hb Adana, cd 30 delGAG,) were analyzed by PCR and reverse dot-blot methods, and α-thal mutations were identified in 150 cases (63.8%). The following genotypes were observed: -$\alpha^{3.7}$/αα (91 subjects), -$a^{3.7}$/-$a^{3.7}$ (24), --^{MED}/aa (9), -$a^{3.7}$/-$a^{4.2}$ (3), -$a^{3.7}$/--^{MED} (1), -$a^{4.2}$/--^{MED} (1), -$a^{4.2}$/aa (9), -$(a)^{20.5}$/aa (2), a^{CS}a/aa (8), a^{CS}a/a^{CS}a (1) and -$a^{3.7}$/a^{CS}a (1). In 85 individuals none of these mutations was found. Our study shows that the -$\alpha^{3.7}$ single gene deletion is a very frequent cause of microcytic, hypochromic anemia in Iran, but other mutations such as Hb Constant Spring, --^{MED}, -$\alpha^{4.2}$ and -$(\alpha)^{20}$ also seem to have acceptable frequencies.

P0553. Molecular analysis of 66 cystic fibrosis carriers in Iranian population.

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Abstract

Identification of mutations causing cystic fibrosis in the Iranian population is essential for assessment of the molecular basis of CF in Iran and the development of strategies for prenatal diagnosis and genetic counseling. In this study, we report the mutations found in the 66 Iranian CF carriers with Heteroduplex analysis on MDE gel in 10 exons of CFTR gene. This study revealed a total of 36% mutations. DelF508 was less frequent in Iranian population than in the European one. The Iranian CF mutations are compatible with the Mediterranean mutation panels.

P0554. *GJB2* Mutations and the Δ (*GJB6*-D13S1830) Deletion as a Cause of ARNSD in the Kurdish Population

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Hearing impairment affects approximately 1 in 1000 neonates. In half of these cases, the deafness is inherited, with mutations in *GJB2* making up the largest fractional contribution in many world populations. *GJB2* encodes Connexin 26 (Cx26), a gap junction protein expressed in non-sensory epithelial cells and the connective tissue cell gap junction system in the inner ear. The putative role of Cx26 connexons is in maintaining the potassium concentration in the scala media. We studied 86 Kurdish families segregating severe-to-profound autosomal recessive non-syndromic deafness (ARNSD) to determine the frequency of *GJB2* and Δ(*GJB6-D13S1830*) mutations in this population. Mutation screening was performed by allele-specific PCR, followed by 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) and one mutation in exon 1 (IVS1+1G>A). No person carried the Δ(*GJB6-D13S1830*) mutation. Fourteen individuals carried the 35delG allele (7 35delG homozygotes, 7 35delG heterozygotes), which accounted for 62.5% of mutant alleles. In decreasing order of frequency, other mutations like R32H, delE120 and IVS1+1G>A have a causal role in deafness in this population, with frequencies of 12.5%, 9.4% and 9.4%, respectively. The IVS1+1G>A mutation is relatively uncommon in other populations and has not been previously identified in Iran. Based on these data, *GJB2* mutations account for approximately 22% of severe-to-profound congenital deafness in the Kurdish population in Iran.

P0555. A novel 9 bp-deletion in the *filamin A* gene results in an OPD-spectrum disorder with a highly variable phenotype

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Frontometaphyseal dysplasia (FMD; MIM#305620) is an X-linked dominant osteochondrodysplasia characterized by prominent supraorbital ridges, defective dentition, micrognathia, hearing loss, joint contractures, and undermodeled metaphyses of long bones. Phenotypic similarities between FMD, the otopalatodigital syndromes types 1 and 2 (OPD1 and OPD2), and Melnick-Needles syndrome (MNS) have prompted speculations that these conditions might be allelic. Recently, it has been shown that in-frame rearrangements in the *filamin A* gene (*FLNA*), encoding a protein involved in actin reorganization, are responsible for the four phenotypes, now called collectively "OPD-spectrum disorders". The mutations identified so far are clustered in five exons, encoding the actin-binding domain and rod domain repeats 3, 10, and 14/15. To date three FMD mutations have been reported in exons 22 and 29 (rod domain repeats 10 and

14, respectively). Here we report the largest pedigree described to date that consists of 6 affected females and two affected males in four generations. While females show FMD-typical symptoms of variable expression, affected males die soon after birth or during the first years of life and present with a phenotype that overlaps with severe OPD2 and MNS (including kidney and urinary tract anomalies). In the family reported here, the disease is caused by a novel in-frame deletion of 9 base pairs predicting the exclusion of three amino acids in the rod domain repeat 14 that confirms the assumption that this part of the *FLNA* gene determines "susceptibility to FMD".

P0556. Δ (*GJB6*-D13S1830) is not a common cause of deafness in Iran.

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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 fraction of patients with *GJB2* mutations have only one mutant allele, and in some familial cases with linkage to the *DFNB1* locus no mutations in *GJB2* are reported. Recently, a large deletion involving the *GJB6* gene encoding connexin 30 which is also located at the *DFNB1* locus (Δ (*GJB6*-D13S1830)) has been reported to cause ARNSD in homozygotes for this mutation and in compound heterozygotes carrying deafness-causing allele variants of *GJB2* on the opposite allele. To evaluate the importance of Δ (*GJB6*-D13S1830) in the Iranian population, we screened 127 deaf probands with ARNSD from various regions of the country. In 115 probands the *GJB2* alleles were normal, the remaining 12 were heterozygote for only one *GJB2* mutation. Screening for Δ (*GJB6*-D13S1830) was completed using PCR primers that amplified the breakpoint junction of this deletion (NEJM 2002; 346: 243-9). Δ (*GJB6*-D13S1830) was not detected in our subjects, suggesting that this mutation is not a common cause of non-syndromic deafness in Iran.

P0557. Detection of rare and novel ß-tathalassemia mutations in the Iranian population

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Beta-thalassemia, by its high freguency and hetrogeneity, constitues a major health problem in Iran. It is the first priority for the Health Ministry among genetic disorders. Prevention of ß-thalassemia requires knowledge of the molecular spectrum occuring in the population at risk. This knowledge is necessary when a prevention protocol is applied to a multiethnic population. Thus the detection of all mutations of ß-thalassemia in each population is the major goal in prevention, and is especially helpful for prenatal diagnosis. Up to now more than 200 different mutations in the ß-globin gene have been reported. About 13 mutations encompass 70 to 90 percent of the mutational spectrum in Iran.These mutations are called the common ß-globin mutations in Iran. The other 10-30% are rare or unkown. In this study six mutations of the codon IVSI-130(G>C), Fr16(-C), codon35(-C), fr23/24(-G), codon8(+G) and codon 20(GTG>GAG) are recognized and added to the spectrum of ß-globin mutations in Iran, using ARMS/PCR and DNA sequencing. The latter 3 cases are reported for the first time in the world.

P0558. SNP genotyping and association analysis of several candidate genes of sporadic Creutzfeldt-Jakob disease identified by expression profiling

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The aetiology of sporadic Creutzfeldt-Jakob disease (sCJD), the most common form of the human prion disease, remains obscure. The sole described genetic factor influencing the susceptibility for this disease is a common polymorphism in the coding region of the *PRNP* gene at codon 129. However, further major genetic predisposing factors for sCJD are still unknown. Therefore, the aim of this study was the identification of informative polymorphisms additional to *PRNP*_129 in candidate genes using an association study in the largest case-control study of sCJD.
We identified several candidate genes which were differentially expressed in sCJD cases performing expression profiling in humans and mice. These candidate genes included intracellular proteases, metal/free radical chelating proteins, calcium binding proteins, growth factors and high density lipoproteins. Thus their physiological involvement in prion diseases was highly probable.
By means of MALDI-TOF MS technique (*MassArray,* Sequenom, San Diego) we genotyped 30 SNPs in eight candidate genes. Genotyping was performed in 584 sCJD cases and 749 healthy controls matched for age and gender which were taken from a population based study performed in the city and region of Augsburg, Germany (KORA Survey 2000). The role of these SNPs as possible risk factors was evaluated. Using this unique patient sample, we could evaluate published data in *PRNP* and other genes which were derived from much smaller cohorts and produced conflicting data.

P0559. A novel 12 base deletion in the cartilage oligomatrix protein gene (COMP) seems to be causative in pseudoachondroplasia: molecular, radiologic and clinical findings in a 5 y old girl

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Pseudoachondroplasia (PSACH) is a dominantly inherited osteochondrodysplasia characterised by short limb dwarfism and joint hypermobility with normal face and intelligence. Growth retardation is rarely recognized before the second year of life. To date, all cases of PSACH have been ascribed to mutations in a particular region of the COMP gene. We report on a 5 y old girl, who was macrosomic at birth due to maternal diabetes mellitus. Heigth was still on average aged 1 year, later on growth velocity declined to the 3rd percentile associated with increasing dysproportionate aspect, accentuated lumbar lordosis, unusual plump and short hands and hyperextensible joints. Diagnosis of PSACH was clinically suggested and further supported by X-ray findings. A very consistent radiologic sign was an anterior protrusion of the central aspects of the vertebral bodies called "tonguing". Molecular analysis of the most likely affected exons 8-19 of the COMP gene revealed apart from two clinically irrelevant polymorphism an novel 12 base deletion del nt 1528-1539 (Δ Ala Asp Lys Val) as an apparently *de novo* heterozygous mutation, which was neither found in the parents nor in her healthy brother therefore being highly likely causative for PSACH. Recurrence risk, although low, could not be completely excluded because of the possibility of germline mosaic, which seems to be the probable genetic background for the recurrence in sibs of unaffected parents. It is worthwhile to mention that also some cases of multiple epiphyseal dysplasia are caused by COMP mutations suggesting a continuous phenotypic spectrum with both conditions enclosed.

P0560. Molecular basis of oculo-cutaneous albinism type 1 (OCA1)in Lebanon

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Oculocunatenous albinism type 1 (OCA1) results from mutations in the tyrosinase gene which lead to partial or complete loss of enzymatic activity. A large number of mutations have been identified worldwide, providing insight into the pathogenesis of the disorder. We performed ophthalmic and dermatological exams on 30 Lebanese subjects with oculocutaneous albinism, then screened for mutations in the tyrosinase gene in an effort to establish the molecular basis of the disorder in our population and compare phenotypic findings. The 5 exons of the gene together with the exon-intron boundaries and part of the promoter region were sequenced. Mutations were found in a total of 13 patients (43%), while no mutations were identified in the sequenced regions in 57% of patients. Fourteen different mutations were identified, of which 8 were novel, while 6 had been previously reported. Mutations were mainly seen in patients with clinical findings suggestive of OCA1A, therefore the absence of mutations in at least some of the other patients may indicate the involvement of other genes

P0561. Inflammatory genes expression in focal myositis and polymyositis using human microarrays

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Objective: The purpose was to identify genes that are differentially expressed in skeletal muscle of patients with focal myositis (FM) and polymyositis (PM), searching for a molecular hallmark responsible of the restricted inflammatory phenomenon in FM.
Methods: Microarray experiments were performed using amplified RNA isolated in muscle specimens from five patients each with FM and PM, and five normal controls. A GeneChip microarrays panel of 160 human inflammatory genes was used.
Results: 58 genes showed significantly increased expression in both FM and PM. They included genes involved in immune response, T-cell cytotoxicity, extracellular matrix breakdown, propagation of the inflammatory process. We found higher activation of metalloproteinases (MMP) 9,12,13 in PM versus FM, and interpheron gamma (IFN-gamma) in FM versus PM. Adhesion molecule ICAM-1 resulted abundantly expressed only in PM. Mn super-oxide-dismutase, inducible nitric oxid synthase and interleukin-1-beta (IL-1 beta) were overexpressed only in FM.
Discussion: Microarray analysis is an effective tool for identifying genes differently involved in the inflammatory/immune response of skeletal muscle during FM and PM. Exaggerated expression of MMP-9, 12,13, and ICAM-1, facilitating lymphocyte adhesion and enhancing T-cell mediated cytotoxicity by degrading extracellular matrix proteins, could contribute to extend inflammatory process in PM. Conversely, selective upregulation of some genes seems to characterise FM. These findings may have practical implications in considering therapeutic different approaches in inflammatory myopathies.

P0562. Analysis of the *SMN* and *NAIP* genes in Brazilian Spinal Muscular Atrophy Patients.

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Spinal muscular atrophies (SMAs) are the second most common neuromuscular disorders in childhood, with an incidence of 1/10.000 births. SMAs are inherited motor neuron degenerative disorders that cause progressive muscular weakness, with high mortality. Three types of SMA studied type I, which is the most severe form, type II or intermediate form, and type III. They can be distinguished according severity, affected muscles or age at onset. There are two genes linked to SMA: the survival motor neuron (*SMN*) and neuronal apoptosis inhibitory protein (*NAIP*). The objective of this study is the molecular diagnosis of these genes in 26 SMA patients, 11 parents and 2 asymptomatic brothers from the Pediatrics Institute of Rio de Janeiro; 40 non-related blood donors from the University Hospital (control sample) and 40 myotonic distrophy patients from the Neurology Institute. Nested PCR was used for both exons 7 and 8 of *SMN* gene, frequently deleted in SMA patients, followed by digestion and 12% poliacrilamide gel electrophoresis. For exons 5 and 6 of *NAIP* gene, PCR was performed followed by 2% agarose gel. In all groups but SMA patients, no deletions were observed neither in exons 7 and 8 of *SMN* gene, nor in exons 5 and 6 of *NAIP* gene. This study showed a high frequency of deletions in SMA patients (88%), and 32% in *NAIP* gene. It was not possible to avoid SMA diagnosis, even in those patients without deletions. DNA study represents an efficient confirmatory test for SMA patients, demanding a non-invasive sample.

P0563. Mutations in the First 90 Residues of the Alpha1(I) Collagen Chain Cause Combined Ehlers-Danlos and OI by Interference with N-propeptide Processing

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Patients with OI/EDS form a distinct subset of osteogenesis imperfecta patients. In addition to skeletal fragility, they have symptoms of Ehlers-Danlos Syndrome. We identified 7 children with OI/EDS and investigated the mechanism of the combined disorder. The probands have OI types III or IV, plus severe large and small joint laxity and early progressive scoliosis without vertebral compression. In all probands, we delineated mutations in the first 90 residues of the α1(I) collagen chain.
Processing of proband procollagen by N-proteinase is impaired. Only 25% of proα1(I) with exon 7 mutations and 65-85% with exons 8-11 mutations was cleaved *in vitro*, despite an intact N-proteinase cleavage site. Pericellular procollagen processing was also delayed. The resulting pN-collagen incorporated efficiently into matrix deposited in culture by osteoblasts and fibroblasts. pN-α1(I) is especially prominent in newly incorporated and immaturely cross-linked fractions of osteoblast matrix. Dermal collagen fibrils of all probands have significantly reduced cross-sectional diameters, as in EDS VII. In differential scanning calorimetry, collagen containing these mutations has a lower Tm than the corresponding procollagen, which is anchored by the N-propeptide. This implies that the mutations interfere with pN-cleavage by disrupting a high-stability region at the N-terminus of the helix and unfolding the secondary structure of the adjacent N-proteinase cleavage site. Thus, the OI/EDS combined phenotype results from mutations in α1(I) which directly cause bone dysplasia and indirectly cause EDS. The EDS component results from incorporation of collagen molecules with retained N-propeptide into matrix in culture and *in vivo,* as in EDS VIIA.

P0564. Screening of families with autosomal recessive non-syndromic hearing impairment (ARNSHI) for mutations in GJB2 gene in Iranian Persian population.

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Mutations in the connexin 26 (Cx26) gene (GJB2) are a common cause of hereditary hearing impairment. The GJB2 mutant allele, 35delG, has been found to have a high prevalence in most ethnic groups. We analyzed 245 Persian families from different provinces in Iran with autosomal recessive non-syndromic hearing impairment.

One proband from each family was initially screened for 35delG mutation using allele-specific PCR primers. The smaples that were heterozygous or negative for 35delG mutation were analized by DHPLC and direct sequencing. Only 34 patients (13.9%) had GJB2-related deafness. Overall, in descending order of frequency, mutations found in the patients studied were as follows: 35delG, V153I, R127H, W24X, delE120, -3170G>A, R184P, 310del14, 314del14, V27I, V27I+E114G, 167delT, 329delA, I69I. The V153I and V27I variations are polymorphisms, 329delA and I69I are unknown mutations. The low frequency of GJB2 mutations in the Persian deaf population is not in agreement with most of the studies reported from other contries, which implies the contribution of other loci in the Iranian Persian deaf population.

P0565. Influence of marriage distance on Pregnancy Loss

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The aim of work was to study the influence of marriage distances in Kaluga Region on pregnancy loss.
Six types of marriages were revealed: evident blood relationship; sympatric marriages-married couple of one nationality originated from one or from neighboring villages (no more than 10 km.); intraprovincial marriages-married couple of one nationality from not neighboring villages (more than 10 km) of one rural region; interprovicial marriages-married couple of one nationality from different provinces of one region; interregional marriages-married couple of one nationality from different regions and international marriages.
Selection of families with Pregnancy Loss is presented in that way: families with one miscarriage - Pregnancy loss (PL) mode up 391 families, with two and more miscarriages Usual pregnancy loss (UPL) - 471. Control -272 families.
The comparison of miscarriage types in the families with PL and UPL according to He-Test, showed that the selections statistically don't differ ($p < 0,002$). This fact allows to combine the studied groups and to analyze them in comparison with control jointly.

Types of marriages	PL + UPL (n=862)		Control (n=272)	
	n	%	n	%
1. evident blood	5	0,58	-	-
2. sympatric	23	2,67	5	1,84
3. intraprovincial	83	9,63	31	11,40
4. interprovicial	304	35,27	95	34,93
5. interregional	355	41,18	118	43,38
6. international	92	10,67	28	10,29
He-Test	0,978			

Sufficiently high comparison of family marriage structure with PL and the control enables us to assume that marriage distances in population being studied is not an essential factor influencing on PL frequency.

P0566. Molecular testing for spinocerebellar ataxias in Poland: five years of experience.

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The hereditary, late onset, spinocerebellar ataxias are clinically and genetically heterogeneous group of neurodegenerative disorders, all characterized by variety of symptoms, including progressive ataxia and dysarthria resulting from degeneration of the cerebellum, brain stem and spinocerebellar pathways. These disorders exhibit also the anticipation phenomenon: an increase of severity and earlier onset of the disease in successive generations.
Over 15 SCAs types are known, the most common are SCA 1, 2, 3, however their geographical localization is diversed, depending on ethnical origin and founder effect.
Molecular diagnostics of SCAs due to dynamic mutations has been carried on in the Department of Genetics in the Institute of Psychiatry and Neurology since 1998, and now molecular tests are available for 7 types of SCAs: SCA 1, 2, 3, 6, 7, 12 and 17. During 5 years we have detected 3 types of autosomal dominant ataxias: SCA 1, 2 and 17. Genetic analyses, both symptomatic and predictive testing embraced over 711 individuals.
In 202 pathogenic expansions of CAG repeats were detected in loci specific of different types of SCA. Among those 149 were manifesting symptoms of ataxia and 52 were in preclinical stage of the disease; in one case prenatal test was performed. Affected subjects are grouped in 83 pedigrees: 68 SCA1, 14 SCA2 and 1 SCA17 (overall group comprises all cases recorded in Poland).
Moreover, in 5 pedigrees, we came across an increased CTG repeats number within *SCA8* gene.

P0567. Analysis of exonic polymorphisms leading to exon skipping in the cystinuria gene SLC7A9

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Cystinuria is a common inherited disorder of renal reabsorption of cystine and dibasic amino acids that results in cystine urolithiasis. The disease is caused by defects in the heterodimeric amino acid transporter rBAT/$b^{0,+}$AT localized in the luminal membrane of renal tubules and intestine. While mutations in the SLC3A1 gene cause cystinuria type A, patients with cystinuria type B carry mutations in the SLC7A9 gene. However, the role of further genes and modifying factors in the aetiology and clinical manifestation of cystinuria has been postulated. We recently demonstrated that the allelic distribution of specific variants in the SLC7A9 gene is statistically significant different between cystinuria patients and controls. Based on this observation we designed minigene approaches to determine the role of the variants in exons 5 and 6 of SLC7A9 in slicing processes. COS7 cells were transfected with expression constructs containing different haplotypes, cDNAs derived from the resulting mRNAs were analyzed. Thereby we could demonstrate that the different alleles cause alternative splicing by exon skipping. Two of the analyzed variants are located within exonic splicing enhancers. We therefore hypothesise that the SLC7A9 polymorphisms in exons 5 and 6 influence the gene expression level of SLC7A9 and may therefore account for the variable penetrance of mutations in this gene.

P0568. Molecular detection of SMNt deletion in SMA patients in Iranian population over a six-year period

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder. One of the candidate genes for this disease is survival motor neuron gene (SMN) that exists in two nearly identical copies, telomeric SMN (SMNt) and centromeric SMN (SMNc). The two genes differ in their exons by only two base pairs, one in exon 7 and one in exon 8 that allow them to be distinguished by restriction site assay.
DNA extracted from blood samples was amplified by PCR. The PCR products were digested by restriction enzymes, *Dra*I and *Dde*I, and subsequently analyzed by polyacrylamide gel electrophoresis followed by silver staining. The ratios of band intensities (SMNt/SMNc) were indicative of carrier (heterozygous deletion of SMNt) or affected (homozygous deletion of SMNt) status of the samples.
Over a six-year period, we have studied 128 clinically diagnosed SMA families for exon 7 and exon 8 deletions in SMNt gene. All of these families were referred for carrier detection and 47 families were also referred for prenatal diagnosis. We have been able to determine the carrier status of 112 families of 128 families referred for carrier detection. In 14 families we could confirm the carrier status only in one of the individuals. A total of 47 prenatal diagnoses were

carried out, of which, 28, 7 and 9 were carrier, affected, and normal respectively. In 3 cases we could not determine whether the fetuses were trait or normal, and they were reported as Trait/normal.

P0569. A novel mutation (R334X) in SMARCAL1 gene in a patient with Schimke Immuno-osseus Dysplasia (SIOD)

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SIOD (OMIM # 242900) is a rare autosomal recessive disorder characterized by the combination of spondyloepiphyseal dysplasia, a peculiar clinical phenotype, numerous lentigines, slowly progressive immune defect and an immune-complex nephritis. SIOD is characterized by a marked variation in severity, ranging from in utero onset of growth retardation and death early in childhood to a milder course with onset late in the first decade or early second decade of life (juvenile type). In approx. seventy percent of SIOD patients mutations within the SMARCAL1 gene (SWI/SNF2-related, matrix associated, actin-dependent regulator of chromatin, subfamily a-like 1) have been found (Boerkhoel CF et al., Nat. Genet. 30: 215-220, 2002). We report about a 6 year old boy, the first child of non-consanguineous parents, with disproportionate dwarfism, nephrotic syndrome, neutropenia, a T-cell-immunodeficiency, lentigines and an atrial septal defect. Intrauterine growth retardation was diagnosed sonographically. He was born per caesarian section at week 36 of gestation with a weight of 1390g, a length of 43cm and a head circumference of 27,5cm. The karyotype was 46,XY. According to clinical findings the diagnosis of SIOD was considered. Direct sequencing of both sense and antisense strands of the 2 noncoding and 16 coding exons of SMARCAL1 showed compound heterozygosity for mutations in exon 3 and 4. The mutation in exon 3 causes a missense error (F279S) and has been found already in other SIOD patients. The mutation in exon 4 causes a premature termination (R334X) and is a novel mutation among patients with SIOD.

P0570. INNO-LiPA MBL2: a validated genotyping assay for mannose-binding lectin (MBL2)

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There is a growing interest in mannose-binding lectin (MBL2) due to its role in innate immunity. MBL2 is a pattern recognition molecule capable of binding to the surface of a broad range of microorganisms. MBL2 activates the complement system through the action of MBL-associated serine proteases (MASPs) leading to opsonization. Several DNA polymorphisms have been characterized, six of which are known to result in considerable variations in the quantity [-550G>C, -221G>C, +4C>T in promoter and 5'UTR] and functionality [R52C, G54D, G57E in exon 1] of MBL2 in serum. A deficiency of soluble MBL2 increases susceptibility to infection and may constitute a significant risk factor when immunity is additionally compromised by another primary defect or by secondary immune deficiency. For example, MBL2 can affect the course of cystic fibrosis (CF) where it has been demonstrated that mutations in MBL2 reduce survival of CF patients.
A rapid assay was developed to genotype these 6 variations. The INNO-LiPA MBL2 test comprises one multiplex amplification followed by reverse hybridization on a strip with 12 specific probes. The assay was validated internally on blood and buccal cell samples. Allele and haplotype frequencies in a healthy, Caucasian population were calculated, also in-house. An external validation study on sixty blood samples with well-characterized MBL2 genotypes (PCR-SSP/real-time PCR) including the 7 commonly found MBL2 haplotypes, was performed. Full agreement with the reference results was obtained. This novel assay which is easy and accurate, will allow MBL2 genotyping/haplotyping of large patient cohorts in any molecular lab.

P0571. A Novel ATP2A2 mutation in a Jewish family with Darier's disease

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Darier's disease (DD) is an autosomal dominant skin disorder characterized by loss of adhesion between epidermal cells and abnormal keratinization. The disease has a world wide distribution with a varied prevalence. Defects in the sarco/endoplasmic reticulum Ca^{2+} ATPase type 2 underlie DD. The encoding gene ATP2A2 is positioned on chromosome 12q23-24 and has 21 exons. To date 140 mutations had been identified in familial and sporadic cases of DD. The aim of this study was to identify the genetic defect in a Jewish family with DD: affected mother and healthy twins (3 months). Genomic DNA was extracted from peripheral blood and PCR was carried out to amplify the exons of the ATP2A2 gene, including the flanking intron regions. PCR products were subjected to automated sequencing (ABI 3100 Avant Genetic analyzer). Taq I restriction analysis was carried out on 3% agarose gel.
A change of C to T at position 391, altering amino acid Arginine 131 to stop codon (R131X) in exon 5 was identified in the mother and the pre-symptomatic twins. The change caused an elimination of Taq I restriction site. The mutation was not present in 50 healthy individuals of the same ethnic origin.
A novel mutation in three members of a family was identified. This is the third mutation found in codon 131. The first: G392A in three patients, two of the same family and G392T was a sporadic case. This codon is located in a highly conserved domain in the cytoplasmic ß–strand domain.

P0572. ‚Usherin mutations in autosomal recessive Retinitis Pigmentosa Spanish families'

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The USH2A gene has been shown to be associated with Usher type II and recent data indicate that non-syndromic autosomal recessive Retinitis Pigmentosa (ARRP) could also be caused by mutations in this gene.
In an earlier work, we identified the C759F mutation in 6 out of 196 ARRP families. The mutational analysis of these cases was extended to the complete coding region of the USH2A gene. Missense, nonsense, frameshift and splice mutations were found in these families (1).
We studied 85 unrelated non-syndromic ARRP patients without the C759F mutation allele.
All the 21 coding exons of the USH2A gene were amplified from the leukocyte DNA. Amplified fragments were scanned using SSCP and direct sequencing. We identified three different mutations in the USH2A gene: 544_6delAA; 2299delG and G713R.
These mutations in USH2A gene were identified, in heterozygous state in 4 patients. In neither of these patients was a change in the other allele found. Segregation analysis confirm the pathogenecity of these variations.
Based on these data, USH2A mutations were found in 4,7% of cases with autosomal recessive Retinitis Pigmentosa.
The wide range of phenotypes associated with Usherin mutations highlights the increasing complexity of the relationship between pathogenetic mutations and disease phenotype.
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P0573. FKRP (826C>A) frequently causes limb girdle muscular dystrophy in German patients

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A novel gene (FKRP) was found to be responsible for both a form of congenital muscular dystrophy (MDC1C) and a form of limb girdle muscular dystrophy (LGMD2I). Our work aimed to investigate the frequency of FKRP (826C>A) in a large cohort of LGMD patients and to correlate these findings with the clinical phenotype.
We present clinical and genetic data of 20 LGMD2I patients from 19 unrelated families. Neurological examination, diagnostic muscle biopsies including immunohistochemistry and sequence analysis of FKRP were performed in this cohort.
Interestingly, one single point mutation (826C>A) leading to an amino acid exchange (Leu276Ile) was associated with a relatively benign clinical phenotype. We identified the previously described FKRP mutation (826C>A) in 20 patients. Thirteen patients were homozygous for the mutation and seven were compound heterozygous. In all patients heterozygous for the 826C>A mutation, a second heteroallelic mutation was detected; two mutations had been described before, four mutations are novel. Interestingly, muscle pain and myoglobinuria were the earliest presenting symptoms in most patients.
In summary, LGMD2I is frequently caused by a single missense mutation of the FKRP gene, namely 826C>A (Leu276Ile). This may simplify the diagnostic workup for LGMD patients of European origin.

P0574. *DAZ* gene diversity and genomic plasticity in AZFc: Structural artwork functional for human spermatogenesis

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Today, AZFc deletions in Yq11 are known as the most frequent worldwide genetic lesion causing male infertility. They are a genomic disorder produced by intrachromosomal recombinations between large repetitive sequence blocks called amplicons. Different amplicons organised in large palindromic structures form the entire AZFc sequence (4.5 Mb). Therefore, it can be predicted that this Y region has a high genomic plasticity with multiple genomic rearrangements and its gene content is probably functionally redundant. We addressed this question with respect to the multi-copy *DAZ* gene family in AZFc, analysing its putative functional redundancy by studying its diversity in men with normal fertility and with infertility. For this purpose we established single nucleotide variant (SNV) markers spanning 3 Mb of the genomic AZFc sequence, especially around the 4 *DAZ* gene copies. In fertile men we identified 17 AZFc haplotypes and linked them to specific Y chromosomal lineages (*Am. J. Hum. Genet. 74:180-187, 2004*). Genetic redundancy was found for *DAZ3* and *DAZ4* genes, which were both deleted in all men belonging to Y haplogroup N. Our results suggest that in humans, the *DAZ* genes are functional redundant and the AZFc sequence now published in GenBank has multiple genomic variations. Since the most recent ancestor of the N haplogroup lived ~10,000 years ago, it is an ancient and successful Y lineage, common throughout northern Europe and Asia. Great care must therefore be taken when partial AZFc-Y deletions are discovered in infertile men and their phenotypic consequences for the patient´s fertility need to be predicted.

P0575. DNA analysis of autosomal dominant polycystic kidney disease in Czech families

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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.DNA presymptomatic diagnosis is performed in our laboratory, using highly polymorphic microsatellite markers for DNA linkage analysis. Presymptomatic DNA diagnosis was performed in 212 unrelated ADPKD families
The direct detection of 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 (36%), the frameshifting mutations in 13 patients (52%) and 3 missense mutations (12%). The mean age of ESRF of PKD2 patients in Czech population was 68 years (from 64 years to 80 years).
The direct detection of mutations in the non-duplicated region of the PKD1 gene was performed in 12 families with linkage to PKD1 gene. We detected two mutations in one family- the insertion of three bases in intron 41 (NT: 11534+2insGGG; AA: IVS41+2insGGG; Gene Bank: L33243) described by Perrichot and new large deletion of 34 bases in intron 42 (NT: 11710-28del34 ; AA: IVS42-28del34; Gene Bank: L33243). The described mutation did not segregate with the disease in the family.
Establishment of localisation and type of mutations and their genotype - phenotype correlation in ADPKD families will improve DNA diagnosis and could help to assess the clinical prognosis of ADPKD patients
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P0576. Do genomic variants in IGF2 and CDKN1C contribute to the etiology of Silver-Russell syndrome?

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Silver-Russell syndrome (SRS) is a heterogeneous syndrome with evidence for a substantial role of genetic factors in its etiology. Apart from other clinical key features, severe intrauterine and postnatal growth retardation are the dominant characteristics of SRS. Therefore, studies on the genetic basis of the disease aimed on genes involved in growth and its regulation. Another key for the identification of (a) SRS gene(s) is the finding of chromosomal disturbances in SRS patients: recently, four growth retarded patients carrying maternally inherited duplication in 11p15 have been described, two of these cases presented SRS-like features. The same region includes the genes IGF2 and CDKN1C and is well known to harbour genomic alterations in patients suffering from Beckwith-Wiedemann syndrome, one of the established imprinting syndromes. Considering these different observations, a growth retardation syndrome in 11p15 as the opposite phenotype to overgrowth is conceivable.
We therefore decided to perform an extensive search of the IGF2 and CDKN1C genes mutations which cause growth disturbances. More than 40 SRS patients were screened for mutations by different detection strategies, allele frequencies of the identified variants were compared between patients and controls.
In case of IGF2, slight differences in the allelic distribution of specific polymorphisms between SRS patients and controls were observed, now analysis of larger cohorts of patients are needed to validate this observation. In CDKN1C, several variants could be detected in both cohorts, but only one patient showed a so far unknown maternally inherited variant; its putatively regulatory function makes further investigations necessary.

P0577. Candidate gene testing for Emery-Dreifuss muscular dystrophy

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Until now two genes, ***STA*** and ***LMNA***, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Scanning 93 patients suffering EDMD or associated phenotypes at the Institute of Human Genetics Greifswald revealed that mutations in ***STA*** and ***LMNA*** together account only for 36 % of the cases. Obviously, further genes are likely to be involved in EDMD. Forced by the lack of families suitable for a classic positional cloning approach we started a functional candidate gene approach.So far we considered such genes as candidates for EDMD that encode (1) functionally related

proteins to emerin and lamin A/C including ***LMNB1***, ***LMNB2***, ***LBR***, ***LAP1***, ***LAP2***, ***NRM*** and ***MAN1*** or (2) proteins interacting with emerin and/or lamin A/C including ***Narf, Zmpste24***, ***BAF***, ***DDX16***, ***PSME3, SREBF1, YT521B***. Additionally, a third group of candidates were those, which are expressed specifically in heart and skeletal muscle – the preferentially affected tissues in EDMD (***FLNC***, ***SMPX***, ***POP 1-3***, ***AKAP 7***, ***Nesprin1*** and ***Nesprin2***). Until now we have studied 22 genes in 95 patients from germany. ***BAF, LAP1, LMNB2*** and ***SMPX*** were proven to be monomorphic. We identified two unique variations in ***DDX16*** (R125Q, T810M), four in ***Nesprin1α*** (29A>G, N323H, V572L, E646K), one in ***SrebF1*** (R812Q), one in ***LAP2*** (E384K), one in ***NRM*** (715C>A) and one in ***FLNC*** (6508C>T), which were not detectable in the reference population. None of the DNA variants have so far been associated to EDMD. To validate these unique variations, *in vitro* mutagenesis and transfection experiments are required to find evidence for a pathogenic effect.

P0578. High mutation frequency in clinically and genetically characterized families with autosomal dominant dopa-responsive dystonia (DYT5a)

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Dopa-responsive dystonia (DRD) is a childhood-onset movement disorder characterized by excellent sustained response to low-dose levodopa. Most DRD is autosomal dominantly inherited with reduced penetrance. In about 50-60% of cases, mutations in the *GTP cyclohydrolase I* (*GCHI*) gene have been detected by SSCP/sequence analysis including many different sequence alterations. Recently, a heterozygous exon deletion has been identified by Southern Blot analysis in a single family. To test for the significance of large heterozygous large deletions/multiplications, we developed a quantitative duplex PCR assay using the *LightCycler*. We report here the results of a systematical mutational screen on 79 samples from 26 typical DRD families using sequence analysis and quantitative duplex PCR (gene dosage studies).
We included 39 patients with clinically typical DRD and 40 unaffected family members from 26 multiethnic families. Index patients had a mean age at onset of 6.9±3.5 years (range: 1-12 years). We detected mutations, including two large exonic deletions, in 81% of the families (21/26). Thirty-three affected and eleven unaffected family members carried mutations, confirming reduced penetrance. Sixteen alterations represent novel mutations and further expand the mutational spectrum in *GCHI*. We found a higher frequency of mutations in *GCHI* compared to previous studies, probably due to rigorous inclusion criteria and a more comprehensive mutational analysis. Exon deletions account for about 10% of mutations in *GCHI*, underlining the importance of gene dosage studies. Interestingly, one family exhibited a deletion of all six *GCHI* exons that would be undetectable by Southern Blot analysis.

P0579. Mutation screening of Clarin-1 and SANS genes in Spanish patients with Usher syndrome

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Instroduction: The Usher syndrome is a recessive hereditary disorder characterized by the association of retinitis pigmentosa, sensorineural hearing loss and vestibular dysfunction.
Clarin-1 and SANS are 2 of the 8 different genes found to be associated to this syndrome to date.
Objective: Mutational screening of Clarin-1 and SANS genes in order to detect the mutations responsible for the disease in Spanish patients with Usher syndrome.
Material and Methods: Genomic DNA of 119 unrelated patients clinically diagnosed of Usher syndrome type I, II or III was extracted from peripheral blood samples.
Clarin-1 and SANS exons and intron/exon boundaries were amplified and directly sequenced. The sequences obtained were compared with consensus sequences in order to detect the presence of any change.
Results: A novel missense mutation in Clarin-1 gene, C40G, was homozygously identified in one patient suffering from Usher syndrome type III.
Four different changes, not previously described, were heterozygously detected in SANS gene: G338R was found in 2 patients; 176C>T, P28L and R346W were identified in other 3 affected individuals respectively. The pathogenic implications of these changes are already not clear.
Conclusions: The genes Clarín-1 and SANS are involved in a very low percentage of Usher syndrome cases in Spain.

P0580. Implications of del35G/GJB2 mutation analysis in evaluation of hearing impairment

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Congenital hearing loss affects 1/500 children, 4% of people younger than 45 years have hearing loss problem, mutation del35G/GJB2 gene being the most common cause of heritable deafness. Considering also the need of early intervention and precise genetic counselling, early detection and exact etiology has become a public health problem. In our study 87 subjects (including 11 newborns diagnosed after neonatal screening) prelingually-deafened and nonsyndromic were analyzed for the presence of del35G/GJB2 mutation, by allele-specific PCR. In 31 cases del35G mutation was found in homozygous status and in 6 cases the mutation was found in heterozygous state. These positive results enable for specific patients the etiologic diagnosis and provide genetic information, but they have also therapeutic implications, the patients with the deletion having the best performance in rehabilitation after cochlear implantation. Molecular analysis has become a routine procedure in diagnosis, differential diagnosis and genetic evaluation of patients with hearing loss. Molecular screening for del35G in GJB2 gene was introduced as component of neonatal screening program for hearing impairment.

P0581. Frequency of EXT1 and EXT2 deletions in hereditary multiple exostoses.

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Hereditary multiple exostoses (HME) is an autosomal dominant bone disorder characterized by the presence of bony outgrowths (exostoses) on the long bones. HME is a genetically heterogeneous condition with at present two causal genes identified: EXT1 on chromosome 8q23-q24 and EXT2 on chromosome 11p11.2. Previous studies have shown that screening of the coding region of both genes in HME patients by standard screening methods such as direct sequencing of PCR products, SSCP or DHPLC results in the identification of a disease causing mutation in approximately 80% of the cases. To increase the sensitivity of HME mutation analysis we optimized the mutation screening protocol for both EXT1 and EXT2. For all coding exons DHPLC conditions were optimized and validated in a large set of 50 HME patients with a known EXT1 or EXT2 mutation. All mutations could be detected under at least 1 DHPLC condition, providing a robust and sensitive alternative for labor extensive and more expensive sequencing analysis.
A set of 20 patients showing no mutation after extended sequence analysis was further analyzed by FISH, MLPA and RNA analysis. Deletions involving the EXT1 gene were detected in 5 patients while part of EXT2 was deleted in 1 patient. One patient showed loss of one EXT1 allele on the RNA level but the underlying cause is still under investigation. These results show that a significant portion of HME patients show large deleterious mutations which are missed by conventional PCR

P0582. Germline BRCA1 5382insC mutation in a couple with breast cancer

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Breast cancer is the most common feminine malignancy worldwide but type of this cancer in couples is rare clinical feature. There are a few study in this issue in the literature. Hereditary breast cancer syndromes can be caused by loss-of-function germline mutations in one of the tumor suppressor genes BRCA1 and BRCA2. The frequency of BRCA1 and BRCA2 mutation carriers in women with breast cancer depend on the study population. The most notable example of founder mutations in these genes is the Ashkenazi Jews, where they have three predominant mutations: 185delAG and 5382insC (in BRCA1) and 6174delT (in BRCA2) seem to account for a substantial proportion of high-risk families. The study of cancer in couples may play important role in the assessment of cancer etiology. Therefore, in the recent study, we examined three predominant mutations of in a couple with breast cancer (wife bilateral, husband unilateral breast cancer) synchronously, their two sons and a sister of husband by allele specific oligonucleotide (ASO)-PCR technique. We detected 5382insC mutation in the couple. However, their sons and sister of husband were not carried any mutation. It has been not reported that a couple with breast cancer is a carrier of 5382insC mutation in BRCA1 gene in the literature, until now.

P0583. 9-base insertion in the MEFV gene in a patient with Familial Mediterranean fever

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Familial Mediterranean fever (FMF; OMIM #249100) is an autosomal recessive disorder, affecting mainly Mediterranean populations and characterized by recurrent attacks of fever and serositis. After cloning of the *MEFV* gene, 43 mutations, including three 3-base deletions and one 1-base insertion, have been associated with clinical phenotypes.
We describe a patient with FMF phenotype and a 9-base insertion within exon 2 in one allele and a polymorphysm in the other allele. Male, 42 y.o., originating from central Italy, reported a 25-year history of short attacks of fever, arthralgias, headache and diarrhoea, associated with increased acute phase reactants and leucocytosis. The complete sequencing of *MEFV* (Gene Bank Access number XM_007971) showed a frameshift in exon 2 in the propositus and his mother and a G>A substition at codon 605 (R202Q) in the propositus and his father. Both parents, however, are asymptomatic.
Gene cloning from nucleotide position 92 to 270 was performed by Topo-TA cloning kit. Subsequent sequencing showed the insertion to consist of 9 bases (GAGGGGAAC) from codon 390. The 9-base insertion causes the insertion of 3 amino-acids (glutamic acid, glycine and asparagine) in position 131, 132 and 133, respectively, of the protein product (pyrin).
Based on this molecular evidence and after the exclusion of other causes, we started colchicine (1,5 mg/die) with success.
Our hypothesis is that the insertion can cause either a reduced level of transcript or else interfere with the biological activity of pyrin. The R202Q could act as facilitator of these effects. Expression studies are underway.

P0584. Hepatic involvement in hereditary hemorrhagic telangiectasia (HHT, M.Osler) is associated with mutations in the *ACVRL1* gene

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Morbus Osler, or hereditary hemorrhagic telangiectasia (HHT), is a heterogeneous inherited disorder characterized by multi-systemic vascular dysplasia and wide variation in its phenotypic expression. Hepatic manifestation is seen in about 8 - 30 % of the patients. The molecular basis for liver involvement is unknown. To assess the genotype/ phenotype correlations concerning liver involvement in HHT, we screened the two known HHT disease loci, the *ACVRL1* and *ENG* genes, for mutations in a clinically well- characterized group of HHT patients with or without liver involvement.
We identified mutations in the *ACVRL1* gene in eight out of 10 HHT patients with hepatic manifestation. No mutations could be identified in the remaining two HHT patients with liver involvement. Among nine HHT patients without liver involvement, four had mutations in the *ACVRL1*, and three in the *ENG* gene. In this study, we have identified six novel *ACVRL1* and two *ENG* disease-causing mutations. We conclude that hepatic manifestation in HHT patients is associated with mutations in the *ACVRL1* gene, but rarely caused through *ENG* mutations.

P0585. Twenty novel mutations revealed by DHPLC analysis of the neurofibromatosis type 1(NF1) gene in southern Italian NF1 patients.

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The identification of mutations in the neurofibromatosis type 1 (NF1) gene has presented a considerable challenge because of the large size of the gene, the lack of significant mutational clustering, the diversity of the underlying pathological lesions and the presence of NF1 pseudogenes. Recently, denaturing high performance liquid chromatography (DHPLC) has been successfully applied to the mutational screening of NF1 yielding mutation detection rates of between 68% and 72.5%. To further testing its suitability in a routine diagnostic setting we evaluated prospectively, by means of DHPLC and DNA sequencing, all 60 exons and splice junction of the NF1 gene in a panel of 85 consecutive, genetically uncharacterized, NF1 patients (43 familial and 42 sporadic cases) from Sicily and Calabria (southern Italy). Germ-line mutations were identified in 64 subjects: twenty of these alterations were novel including two stop codons: c.3574G>T (E1192X) and c.4078C>T (Q1360X); five nucleotide substitutions: c.3327A>C (L1109F), c.3577T>A (F1193I), c.4180A>G (N1394D), c.4193T>A (V1398D), c.6364G>C (E2122Q); five small insertions: c.310_1insTAGCATAAACGATGCTGGTCCAGCA, c.1074_5insGAACCTGCTTTTT, c.4511_2insA, c.6488_9insA, c.6792_3insC ; six small deletions: c.4508delG, c.4625delA, c.5524delA, c.7017delT, c.7084_9delAACTCT, c.7365delT and two splice site mutations c.4269+1G>A, c.7806+1G>A.
None of the novel mutations was detected in 100 control chromosomes from a group of healthy individuals from Sicily and Calabria. These novel mutations contribute to the definition of the germ-line mutational spectrum of NF1. DHPLC confirms to be a rapid, efficient and accurate tool for NF1 mutational analysis.

P0586. Transcriptional and Translational Regulation of the Léri-Weill and Turner Syndrome Homeobox Gene *SHOX*

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Regulation of gene expression is particularly important for gene dosage dependent diseases and the phenomenon of clinical heterogeneity frequently associated with these phenotypes. We here report on the combined transcriptional and translational regulatory mechanisms controlling the expression of the Léri-Weill and Turner syndrome gene *SHOX*. We define an alternative promoter within exon 2 of the *SHOX* gene by transient transfections of mono- and bicistronic reporter constructs and demonstrate substantial differences in the translation efficiency of the mRNAs transcribed from these alternative promoters by *in vitro* translation assays and direct mRNA transfections into different cell lines. While transcripts

generated from the intragenic promoter (P_2) are translated with high efficiencies, mRNA originating from the upstream promoter (P_1) exhibit significant translation inhibitory effects due to seven AUG codons upstream of the main open reading frame (uAUGs).

Site directed mutagenesis of these uAUGs confers full translation efficiency to reporter mRNAs in different cell lines and after injection of *Xenopus* embryos. In conclusion, our data support a model where functional *SHOX* protein levels are regulated by a combination of transcriptional and translational control mechanisms.

P0587. The molecular basis of medium-chain acyl-CoA dehydrogenase deficiency in a Belgian population

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most frequent defect of fatty acid β-oxidation. MCAD deficiency is caused by mutations in the ACADM gene and is inherited as an autosomal recessive disorder. The prevalence of the disease is estimated to be between 1/10000 and 1/20000. One mutation, K329E, is common in most populations.
The aim of this study was (1) to determine the prevalence of the K329E mutation in the general population in Belgium and (2) to identify mutations in patients diagnosed as having MCAD deficiency by analysis of acylcarnitine profiles in newborn screening.
The mutation K329E was found five times in a total of 341 DNA samples of unrelated individuals (carrier frequency 1/68; prevalence of homozygotes 1/18500). Twelve unrelated patients with MCAD deficiency were first studied for the presence of the K329E mutation, and when this mutation was absent or present in only one allele, then the whole gene was sequenced. Seven patients were homozygous for the K329E mutation; four were compound heterozygote K329E and 1102-1105del (2 patients), Y76H or G249R (1 patient each). One patient was homozygous for the 244insT mutation. These mutations, except G249R, have been previously described. In this study the K329E mutation was present in 18/24 (75%) of the alleles. Therefore, the estimated carrier frequency and prevalence are 1/51 and 1/10404. One couple requested prenatal diagnosis (PD), another couple preimplantation genetic diagnosis (PGD). One homozygous normal (PD) and a healthy twin (a homozygous normal and a K329E carrier child) was born after PGD.

P0588. Segmental paternal isodisomy for chromosome 1p: hypophosphatasia due to homozygosity for a paternal heterozygous G403S TNSALP mutation.

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Hypophosphatasia is an inherited disorder characterised by defective bone and teeth mineralization and deficiency of serum and bone alkaline phosphatase activity. Symptoms are highly variable; severe perinatal and infantile forms (HOPS, OMIM # 241500) are transmitted as an autosomal recessive form while milder forms follow both autosomal recessive and dominant mode of inheritance. A wide spectrum of mutations within the TNSALP gene at 1p36.1 have been described in severe and in mild forms. Compound heterozygous missense mutations account for most of the severe cases. We report about a severe form detected sonographically at week 19. After termination the male fetus showed skeletal anomalies of a metaphyseal type of chondrodysplasia compatible with hypophosphatasia. Direct sequencing of the TNSALP coding sequence including exon-intron borders and untranslated exons using chorionic villus DNA identified a 1258G>A mutation (G403S). Sequencing exon 11 revealed heterozygosity for G403S in the father with hypomineralisation of teeth but no mutation in the clinically normal mother. Genotyping both parents and fetus for 20 microsatellites spanning a 205.5cM chromosome 1 segment between D1S228 (1p36.21) and D1S439 (1q42.12) uncovered lack of maternal alleles and the presence of only one paternal allele for all 1p loci distal to D1S2753 (1p21.3, 120cM) but heterozygosity and biparental inheritance for all 1q and 1p21markers. Karyotyping at 450 G-band level and FISH using probe for 1p36 did not reveal any evidence for a deletion. It is concluded that the fetus is homozygous for the paternal G403S mutation due to paternal isodisomy for an at least 32.7Mb 1p-segment.

P0589. Primary congenital glaucoma; molecular study in a sample of Mexican patients

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Primary congenital glaucoma (PCG), an autosomal recessive disorder due to abnormal development of the anterior eye portion, is an important cause of childhood blindness. CYP1B1 enzyme is able to metabolize steroid hormones and participates in tissue development. In the present study, we analyze the CYP1B1 gene of 14 non-related and sporadic patients with PCG. Onset of clinical symptoms ranged from birth to 12 months (mean 4.5 months), the male:female ratio was 10:4 while ocular findings were similar in all patients except for two cases of difficult control. Consanguinity was observed in 3 families. DNA sequencing analysis of the CYP1B1 gene showed no missense or nonsense mutations, only polymorphic changes similar to those observed in normal controls were found to be present. These data allow to conclude that sporadic cases with PCG are not consequence of mutations in the CYP1B1 in our population, at least in the analyzed sample. This is the first study of the CYP1B1 gene in Mexican patients with PCG, this analysis is very important in diagnosis and genetic counseling of PCG in this population.

P0590. *Real-time* PCR SNP analysis in molecular diagnostics of neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1) is one of the most common human autosomal dominant disorders. NF1 has been expressed with various and heterogenous clinical manifestations and serious complications. The gene for neurofibromatosis type 1 (NF1) was mapped to chromosome 17 by positional cloning and has been found to contain mutations in NF1 patients. It exhibits full penetrance and a high mutation rate. Thirty to 50% of NF1 patients represent new sporadic cases and usually new mutations. Molecular analysis and genetic counseling is limited to the identification of the specific mutation in each patient or family or to the use of DNA polymorphisms in *linkage* analysis and detection of large *de novo* deletions. We analyzed 50 families with neurofibromatosis type 1. NF1 was diagnosed clinically according to the NIH criteria. DNA was obtained from peripheral blood of patients and related individuals. We used *real-time* PCR SNP analysis for linkage analysis and detection of large *de novo* deletions in the affected families and individuals. Fifthy families in the Croatian population were studied using three SNP intragenic NF1 markers. The informativities of these three markers were: C_2557613_10 (rs2953014), C_2533294_1 (rs2070733) and C_16032374_10. (rs2107359) 43.3%. The large *de novo* deletions were found in 3 families with the sporadic NF1.

P0591. Phenotype-Genotype relationships in Usher Syndrome type IIA

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Usher syndrome is an autosomal recessive disorder characterised by hearing impairment and retinitis pigmentosa. This syndrome is clinically heterogeneous and three clinical forms have been described: USH types I, II and III. Three genetic subtypes (A, B and C) of USH2 are known, being the USH2A the most frequent. Mutations in the extracellular matrix protein Usherin underlie the

Usher type IIA subtype. To better understand the contribution of Usherin mutations in the spectrum of Usher IIA phenotypes, DNA from 27 patients with a referring diagnosis of Usher II were subjected to the mutational analysis of all coding regions of the USH2A gene. A thorough clinical and audiological analysis was performed in all cases.
Ten different disease-causing mutations were identified: frameshift (c.947_54dup; c.1214del; c.2135del; c.2299del; c.2431_2432del and a large deletion encompassing exons 9-14); nonsense (p.R34X; p.Y506X) and missense (p.E478D; p.C759F). Disease-alleles were identified in 12 out of the 27 families tested (44%).
The absence of vestibular disfunction was observed in all cases. The degree of the congenital and bilateral hearing impairement ranges from moderate to severe. A progressive pattern was observed in some cases in which a profound deafness is observed at the third decade The data presented confirm the observation that mutations in Usherin cause a clinical presentation of hearing loss that is variable among Usher II syndrome patients.
This work was supported by grant FIS (02/0052; EsResNet) and the ONCE.

P0592. Molecular analysis of p28 dynein light chain

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Primary Ciliary dyskinesia (PCD) or Immotile Cilia Syndrome (ICS) is characterized by recurrent infections of the respiratiory system, bronchiectasis and sperm immotility. Moreover, electron microscopy revealed axonemal defects in PCD patients, e.g. the absence of inner and outer dynein arms. This indicates that mutations in dynein genes could be a reason for the disease. To prove this hypothesis, we have started to analyse a gene encoding a dynein light chain (mp28). The murine p28 gene consists of six exons and the genomic range extends about 8 kb. Using RT-PCR approach mp28 expression was detected in several tissues containing cilia or flagella. Moreover, Northern blot analysis demonstrated strong expression of mp28 in male germ cells, starting from postnatal day 15.
Antibody was generated against the mp28 protein to elucidate its location. Our data confirm that murine p28 protein is localized along the entire axoneme of the flagella, indicating its critical role in sperm motility. We therefore hypothesized that the human homologue (hp28) of the mp28 gene would be an attractive candidate gene mutated in patients with ICS or immotile sperm. To strengthen our idea, we designed primers to amplify the exons and flanking regions of the hp28 gene. We analysed 20 patients with immotile sperms using the WAVE technology and identified different polymorphisms within the hp28 gene, some of which resulted in the exchange of amino acids. Furthermore, to analyse the function of this dynein light chain within the axonemal complex, we have started to generate mp28-deficient mice.

P0593. Detection of variability in apo(a) gene control regions.

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High lipoprotein(a) [Lp(a)] level is an independent risk factor for development of premature atherosclerosis. Despite nearly exclusive genetic determination to a unique locus, a gene for apolipoprotein(a) [apo(a)], are Lp(a) levels highly variable among individuals. There is a gene size heterogeneity expressing 40-60% of plasma Lp(a) concentrations. The rest is hypothesized to be determined by differences in apo(a) transcription efficiency. The aim of our study was to determine the rate of polymorphisms occurrence in known apo(a) control regions.
We pooled a sample of individuals with Lp(a) levels over 85 mg/dl and a sample with Lp(a) levels below 5 mg/dl. To find unknown changes in relevant control elements we used DGGE analysis of specifically amplified fragments of proximal promotor and two previously identified enhancers DHII and DHIII. We genotyped STR at a distal promotor to get the whole pattern of sequence variance within sites with described influence on apo(a) transcription.
Beside the two already described changes (+93 C>T; +121G>A) we did not find other common polymorphism within the proximal promoter. Sequence of the DHII enhancer revealed a strong conservation and showed no change occurrence. Nevertheless the DHIII enhancer possess a much higher diversity and could at least to some extent explain the wide range of Lp(a) levels. Our conclusion is there will be another loci with some effect on apo(a) transcription rate. Thus the phenotype is determined by a great number of haplotypes with slightly different impact on apo(a) expression.
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P0594. A new Na+/K+-ATPase mutation causes Familial Hemiplegic Migraine type 2 with cerebellar signs

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Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with hemiparesis during the aura. In over 50% of the cases, the causative gene is CACNA1A (FHM1) which produces a phenotype with cerebellar signs including ataxia and nystagmus. Recently, mutations in ATP1A2 on chromosome 1q23 encoding a Na+/K+-ATPase subunit were identified in four families (FHM2). We now describe an FHM2 pedigree with a fifth ATP1A2 mutation. The phenotype was particularly severe and included hemiplegic migraine, seizure, prolonged coma, elevated temperature, sensory deficit, and transient or permanent cerebellar signs, such as ataxia, nystagmus, and dysarthria. A mild crossed cerebellar diaschisis during an attack further supported the clinical evidence of a cerebellar deficit. This is the first report suggestive of cerebellar involvement in FHM2. A possible role of CACNA1A in producing the phenotype in this family was excluded by linkage studies to the FHM1 locus. The study of this family suggests that absence of cerebellar signs may not be a reliable indicator to clinically differentiate FHM2 from FHM1.
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P0595. A Splice-Junction Mutation In Sbf2 Gene Causes Autosomal Recessive Charcot-Marie-Tooth Disease (cmt4b2) In A Family from southern italy

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Autosomal recessive Charcot-Marie-Tooth disease type 4 (CMT4) comprises a group of clinically and genetically heterogeneous disorders of the peripheral nervous system. Seven genes responsible for autosomal recessive demyelinating CMT have been identified so far.
In this study, we report a small pedigree with a recessive form of CMT (CMT4B) from Southern Italy in which the linkage to chromosome 11q23 was excluded.
The family originated in a small village in southern Italy. There were seven individuals in two generations with two affected subjects. We performed haplotype analysis using highly polymorphic microsatellite markers located on chromosome 11p15. Subsequently, direct sequencing of the *SBF2* gene showed a G→C exon 32/intron 32 splice-junction mutation.
In the current study we identify a splicing mutation in *SBF2* gene in a small family with CMT4B linked to chromosome 11p15. Mutational screening of *SBF2* revealed a homozygous mutation in the splice-junction site of exon 32 (+1G→C) in the affected patients. The variation was also confirmed by digestion with restriction enzyme Alu I, which cleaved the wild-type PCR product of 370 bp into 210 bp and twice 80 bp digestion fragments. The corresponding region of the *SBF2* gene in our affected patients was cleaved into 210 bp, 80 bp, 68 bp and 12 bp digestion fragments. This mutation was absent in 100 control chromosomes examined.

This is the first finding of a mutation in the *SBF2* gene that alters the correct splicing of the gene. Furthermore, these data confirm that mutations in the *SBF2* gene are causative of CMT4B2.

P0596. A new missense mutation in the CYS 2 regulatory domain of PRKCG gene causing Spinocerebellar Ataxia Type 14 in an italian family

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SCA14 is due to point mutations in PRKCG gene, coding for the Protein Kinase C gamma, an isoform of protein kinase C, a family of serine/threonine kinases predominantly expressed in central nervous system. To date four different point mutations have been described causing a dominant non-episodic pure cerebellar ataxia. All of these four mutations were located in the Cys2 regulatory protein domain, whereas mutations in the catalytic domain have been described segregating with a form of retinitis pigmentosa. In order to investigate the frequency of SCA14 among Italian ataxic patients, a screening for mutations of PRKCG gene was performed in a sample of 85 unrelated patients in which repeat expansions in known SCA genes were previously excluded. A family was found carrying a missense mutation of one of the most evolutionarily conserved elements of the Cys2 domain, segregating with the disease. The phenotype included, beyond the slowly progressing cerebellar symptoms, also extrapyramidal signs and cranial nerve deficits. In addition in some cases the disease was characterized by ataxic episodes as in Episodic Ataxia type 2, although mutations of CACNA1A gene were excluded. The present results confirm the clustering of the SCA14 causing mutations in the DAG binding site of Protein Kinase C, and widens the spectrum of the associated highly variable phenotypes.
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P0597. Atlastin1 mutations are frequent in young onsetautosomal dominant spastic paraplegia

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The atlastin1 gene has recently been implicated in SPG3A, a form of autosomal dominant pure spastic paraplegia in 8 families so far. To determine the relative frequency, phenotype and mutation spectrum of SPG3A in patients with pure autosomal dominant spastic paraplegia and onset before age 20, we sequenced the atlastin1 gene in 31 families in which mutations in the spastin gene (SPG4 locus), had previously been excluded. We identified 12 families (39%, n=34 patients) with 9 different missense atlastin1 mutations, seven of which are newly described. The main clinical characteristic of these SPG3A patients was pure spasticity with very young onset (4.6 ±3.9 years) and slow progression. However, additional signs such as decreased vibration sense and wasting in lower limbs, sphincter disturbances and scoliosis were found in a minority of patients. In addition, several gene carriers were clinically affected but still asymptomatic (n=5) or had no clinical signs (n=2), indicating incomplete penetrance. Compared to patients from 43 patients meeting the same diagnostic criteria and 126 patients with SPG4, the major form of autosomal dominant spastic paraplegia, SPG3A patients had earlier onset, less frequently increased reflexes in the upper limbs, decreased vibration sense in the lower limbs or sphincter disturbances, but more frequently observed wasting in the lower limbs and scoliosis. This study enables us to estimate the frequency of the SPG3A mutations in France at 39% in families with young onset autosomal dominant spastic paraplegia after exclusion of SPG4 cases. So far, most mutations have been private.

P0598. Truncating and non truncating mutations of P/Q Ca2+ channel subunit Cav2.1 causing Episodic Ataxia 2 in a large sample of patients

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Episodic Ataxia type 2 (EA2) is mostly due to loss of function mutations truncating or severely disrupting of the pore-forming (Cav2.1) subunit of P/Q type Ca2+ channels, coded by CACNA1A gene. Gain of function missense mutations of the same gene, instead, are responsible for Familial Hemiplegic Migraine. In a few cases EA2 is due to missense or other mutations non-truncating/ disrupting the Cav2.1 subunit. Thirthy-four patients with typical EA2, and 8 with cerebellar ataxia of no known genetic type, are screened for CACNA1A gene mutations. Six new Cav2.1 missense or non-truncating/disrupting mutations have been so far detected and 1 truncating mutation. From almost doubling the number of non-truncating mutations previously known, it becomes clear that they tend to have preferential location in specific protein regions, namely S5-S6 linkers and their borders. Their associated clinical phenotype is comparable to that reported for carriers of truncating/disrupting mutations, but data suggest a possible difference in age at onset and frequency of mental retardation. These results show that EA2 mutations non-truncating/disrupting Cav2.1 subunit are not rare as previously thought. Their associated phenotype might be less severe than that of truncating/disrupting mutations. They are clustering in highly conserved protein regions which must be particularly vulnerable to aminoacid changes and likely to have a critical role in the channel activity.
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P0599. Overview of the molecular diagnosis of facioscapulohumeral muscular dystrophy (FSHD) in Hungarian patients

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FSHD is the third most common inherited neuromuscular disorder and is inherited in an autosomal dominant way. The clinical symptoms are highly variable; the age of onset and the clinical severity differ even within the same family. The locus responsible for the disease was mapped to 4q35. Patients diagnosed with FSHD have a smaller, BlnI resistant EcoRI fragment (≤38kb) in the 4q35 region detected by probe p13E-11 and Southern blot analysis than healthy individuals. Extensive mapping and sequencing work revealed that copies of D4Z4 repeats at the 4q35 locus are between 1-10 in patients whereas unaffected individuals have 11-100 copies. The gene responsible for the FSHD phenotype has not been found yet. Analyses of 52 individuals diagnosed as FSHD and three prenatal cases were completed during the last three years. Out of the clinical assumptions 44 were confirmed by the Southern blot analysis. In eight cases the diagnostic fragments were not detected. In addition, 16 asymptomatic family members of the FSHD patients were included into the study where in one case pathological fragments were detected. In cases where deletion of the p13 region or interchromosomal translocations was suspected, dosage test of 4q35 and 10q26 was performed. Comparison of severity of symptoms with the fragment sizes revealed correlations similar to data published by other laboratories.
Additionally, a case history of a de novo mutation resulting in an 8.5 kb fragment found in monozygotic twins and the results of the prenatal diagnoses will be presented.

P0600. Familial hypoparathyroidismlinked to a GCMB gene mutation

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GCMB is a transcription factor whose defect in mouse results in absent parathyroid glands, hypoparathyroidism, and residual PTH production from aberrant cells in the thymic area. In man the defect was reported in one familial case with undetectable serum PTH. We observed a five year-old boy with severe, symptomatic hypocalcemia. The medical history was unremarkable. The parents were first cousins. An eight year-old sister had presented a cardio-pulmonary arrest at the age of ten months associated with profound hypocalcemia. Two other siblings were in good health. No findings were noted on physical and neurological examination except for a bilateral Chvostek sign. No specific dysmorphia was present. Blood chemistry showed isolated hypoparathyroidism with severe hypocalcemia (Ca = 4.9 mg/dl, P = 10.6 mg/dl). PTH was inappropriately low but clearly detectable (7 pg/ml). 25(OH)vitD was low and 1,25(OH)2vitD low-normal. Karyotype, 22q11 FISH, and Calcium-Sensing Receptor gene sequencing showed no abnormality. SNP analysis showed possible linkage to the GCMB locus. Sequencing the GCMB gene showed a missense Gly63Ser mutation, homozygous in both affected siblings, heterozygous in both parents, and not homozygous in unaffected sibs. This missense was absent from 208 control chromosomes. Functional testing of the mutated GCMB in DF-1 and COS cells co-transfected with a GCM-responsive promoter fused to a luciferase reporter showed loss of transactivation activity despite unaltered DNA-binding activity. This is the second family reported with hypoparathyroidism linked to a GCMB defect. It confirms the presence of detectable levels of circulating PTH as observed in the corresponding mouse KO.

P0601. Inactivating missense and nonsense mutations in the epidermis-type lipoxygenase genes in patients with autosomal recessive congenital ichthyosis (ARCI).

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Autosomal recessive congenital ichthyosis (ARCI) forms a clinically and genetically heterogeneous group of severe hereditary keratinization disorders. Up to the present, five loci for ARCI have been mapped. Mutations in ALOXE3 and ALOX12B on chromosome 17p13, which code for two epidermal lipoxygenases, were recently described in ichthyosis patients from Mediterranean countries. Epidermis-type lipoxygenases (LOX) are preferentially synthesized in skin. Products of ALOXE3 and ALOX12B, 12R-LOX and eLOX-3, are subsequent members of the same pathway converting arachidonic acid via 12(R)-HPETE to the corresponding epoxyalcohol, 8(R)-Hydroxy-11(R),12(R)-epoxyeicosatrienoic acid. Here we describe molecular and clinical findings in 150 families with ARCI originating from Middle Europe, Turkey, India, and Arab countries. We identified 20 novel point mutations of ALOXE3 and ALOX12B, including 17 missense mutations, in 20 families. In ALOX12B, 14 mutations were found in exons 1-3,5-10, and 14. Six mutations were detected in exons 3,5-6,11, and 13 of ALOXE3. To analyse the expression, recombinant mutated genes were expressed in HEK-293 cells, total protein was isolated and incubated with the corresponding substrate, and enzymatic activity was measured. Analysis of reaction products demonstrated that all but two recombinant mutants showed no enzymatic activity. Missense mutations Leu237Met and Arg145Ala in ALOXE3, though, showed regular activity, pointing to coding polymorphisms. Investigation of disease causing mutations in ALOXE3 or ALOX12B and the respective ARCI phenotype did not lead to a clear correlation. Since the function of the epoxyalcohols is still unclear, further biochemical and expression studies in keratinocytes will give a deeper understanding of the pathway leading to ichthyosis.

P0602. Mutation profile of MYO7A gene in Spanish patients with Usher Syndrome type I

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1-INTRODUCTION: Usher syndrome type I (USH1) is an autosomal recessive disorder characterized by profound sensorineural hearing loss, retinitis pigmentosa and vestibular dysfunction. The Myosin VIIA (MYO7A) gene has been reported to be the main responsible for USH1.
2-OBJECTIVE: Mutation screening of the MYO7A gene in Spanish patients with Usher syndrome type I was carried out in order to detect the mutations responsible for the disease in each patient.
3-MATERIALS AND METHODS: Genomic DNA was extracted from peripheral blood from 86 affected individuals corresponding to 58 families. The mutation screening was performed by the use of SSCP analysis of individually amplified exons and intron boundaries. DNA fragments displaying an abnormal SSCP pattern were sequenced.
4-RESULTS:

Mutation	Type	Location	Families
S210X	Nonsense	Motor	1
G214R	Missense	Motor	2
R336H	Missense	Motor	1
A397D	Missense	Motor	1
1614-1615del	Frameshift	Motor	1
C628X	Nonsense	Motor	1
Q821X	Nonsense	IQ	3
K1080X	Nonsense	MyTH4	1
R1168P	Missense	MyTH4	1
E1170K	Missense	MyTH4	2
E1327K	Missense	FERM	1
4039-4053del	Frameshift	FERM	1
L1484F	Missense	FERM	1
T1566M	Missense	FERM	1
Y1719C	Missense	Post-SH3	2
6025delG	Frameshift	FERM	2

5-CONCLUSION: USH1 is caused by a high diversity of mutations in MYO7A, which have been found to be distributed along the entire gene.

P0603. C282Y and H63D mutations of *HFE* gene in patients with hereditary hemochromatosis and in patients with increased risk of hereditary hemochromatosis

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Hereditary hemochromatosis (HH) is a widly-spread hereditary disease, related to iron overload resulting in severe organ damage. It is associated with missense mutations in the *HFE* gene. About 85-90% of HH patients are homozygous for the C282Y mutation; 5-10% are homozygous for H63D or are compound heterozygotes C282Y/H63D. No clear data is available on HH in Russia, though allele frequencies of C282Y: 4.8% and H63D: 12.8% have been reported from.some small East Siberian populations.
We examined 63 HH probands with clinical and biochemical data typical for HH. 44 blood samples were available for the study. These samples were genotyped for the C282Y and H63D mutations: CC - 84.6%; CY - 10.3%; YY - 5.1%; HH - 56.8%; HD - 41.0%; DD - 2.2%. We also studied 609 patients from hepatology, endocrinology and cardiology departments as they were likely to have a high frequency of HH. So far we genotyped 247 for C282Y and 412 for H63D: CC - 93.5%; CY - 6.1%; YY - 0.4%; HH - 69.4%; HD - 27.7%; DD - 2.9%. The low frequency of mutant homozygotes among HH patients can

be explained by other mutations influencing HH. We estimate high frequency of YY genotype in population according to second group results; at the same time we got small number of cases with HH during 2 years of studies; this might mean that YY homozygotes don't necessarily develop typical signs of HH.
We would be very cautious about recommendations to use C282Y/H63D genotyping for routine HH screening or diagnosis.

P0604. Use of residual clinical blood samples for the establishment of genetically characterized cell lines

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To capture rare and valuable diseases for research or diagnostic standards, a strategy of collecting residual blood samples from clinical testing labs was established. These samples were often of significant age (7-14 days), and subsequent transformation for expansion and testing was uncertain. Thirty-three cell lines were established from quantities of blood as small as 1 ml. These samples contained mutations associated with genetic diseases of significant public health importance. The targeted mutation was confirmed in all cell lines through reference testing. Further, all point and deletion mutations tested were stable through approximately 20 population doublings in culture. Of the 33 cell lines, 21 (associated with 11 diseases) were pilot-tested in a simulated performance evaluation program. Samples were sent to at least 5 laboratories, most of which routinely performed clinical testing for the targeted mutation. A total of 25 molecular genetic laboratories tested some cell lines. At least two different molecular technologies were used. Mutations were correctly identified in 246/263 samples (93.4%). Problems were most often associated with DNA isolation steps and/or failed PCR reactions. The number of repeat sequences reported for Huntington disease samples varied somewhat. Other errors appeared to be random. These results suggest that sample development for proficiency testing and performance evaluation should include pilot testing in the field whenever possible. These validated cell lines and/or products derived from them will be available through Coriell Cell Repositories (Camden, NJ, USA; www.coriell.org/ccr) for use in genetic testing quality assurance.

P0605. A repository for neurological gene discovery

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Clearly family history plays a role in many neurological disorders, suggesting genetic influence. Gene discovery has elucidated some Mendelian causes of neurological disease and may reveal general biological mechanisms in those which do not have clear inheritance. Towards the goal of gene discovery, the NINDS has established a repository of DNA samples, immortalized cell lines, and accompanying clinical data for a set of disorders [Stroke, Parkinsons, Epilepsy, Motor Neuron Disease (see http://locus.umdnj.edu/ninds)]. This project is conceptually related to similar initiatives at other institutes at the NIH (Including at NIGMS, NIAMS, NHLBI, and others). Data for each disease are collected using standardized instruments, defined in clinical diagnostic elements (CDEs), which are based on published diagnostic criteria for these disorders. To date, the repository has received samples from more than 1,000 individuals, including 786 with Parkinsonism, 172 with stroke, 20 with epilepsy, and 90 controls. Samples were collected in four foreign countries as well as multiple sites in the United States, providing a diversity in ethnicity. An initiative which allows reimbursement to those collecting samples exists to promote such large scale collection and immediate sharing (http://grants2.nih.gov/grants/guide/notice-files/NOT-NS-03-016.html). In summary, the field of Neurology is ready for complex gene discovery research. A major cooperative effort is required; no single researcher is likely to obtain the large number of samples required for gene discovery. The NINDS Repository will provide the resources needed.

P0606. A novel recurrent PTPN11 mutation in patients with LEOPARD syndrome

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LEOPARD syndrome (LS) is an autosomal dominant disorder characterized by Lentigines and café-au-lait spots, EKG anomalies, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and Deafness. LS is due to specific gain-of-function mutations in the PTPN11 gene, which is mutated in roughly 40% of clinically typical Noonan syndrome (NS) patients. LS shows a strong genotype/phenotype correlation, as only two recurrent, specific mutations (Y279C in the 7th exon and T468M in the 12th exon) have been reported insofar in 13 patients. Those mutations are observed in > 90% of all LS cases.
We report 20 new patients with LS, including 6 patients with Y279C, 8 with T468M, 3 patients with a new, recurrent mutation in exon 13, and 3 patients with no detectable mutations. The new recurrent LS mutation affects the PTP domain in a region in which very few mutations have been reported insofar for NS. This new mutation was not yet reported in classical NS, and was not found in our cohort of over 320 NS cases.
Detailed clincal data on our 20 cases will be shown. A comparison of detailed phenotypic data between the 2 most prevalent mutations will be presented.

P0607. Novel S143F mutation in LMNA presenting with features of muscular dystrophy and progeria

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LMNA gene mutations cause the so-called laminopathies. They have been associated to probably the widest range of phenotypes so far including autosomal dominant Emery-Dreifuss muscular dystrophy (AD EDMD), autosomal recessive Emery-Dreifuss muscular dystrophy (AR EDMD), limb-girdle muscular dystrophy type 1B (LGMD1B), dilated cardiomyopathy with conduction defects (CMD1A), Charcot-Marie Tooth neuropathy (CMT2B), familial partial lipodystrophy (FPLD), mandibuloacral dysplasia (MAD), Hutchinson-Gilford progeria (HGPS), atypical Werner's syndrome (WNR), lipoathrophic, insulin resistant diabetes mellitus, disseminated leukomelanodermic papules, liver steatosis, cardiomyopathy (LIRLLC), autosomal dominant neuropathy with cardiomyopathy and leukonychia and cardiocutaneous progeria. Additionally, several intermediate phenotypes have been described combining features of different entities. Here we report on a five years old female patient presenting in early childhood with muscle weakness and wasting of shoulder girdle and neck muscles. A muscle biopsy showed a myopathic pattern. Later contractures of the pelvic muscles and the spine as well as progeroid features were noted including growth retardation, scleroderma, scalp hair loss, cox valga, pointed nose, dysplasia of the clavicles and phalangeal dysplasia. Mutational analysis of the LMNA gene revealed a novel mutation S143F, that is placed in the gene's region known for mutations associated to atypical WRN. In summary, we present the first patient with a LMNA mutation who bridges the gap between muscular, dysmorphic and progeroid phenotypes. This finding supports the view that laminopathies represent a group of contiguous but clinically extremely heterogeneous diseases.

P0608. Genotype-phenotype correlations in Hungarian spinal muscular atrophy families

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Proximal spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disease. SMA has been subdivided into three clinical phenotypes (SMA I, II, III) based on severity of symptoms. Homozygous exon deletions in SMN1 gene are responsible for all three types of SMA in 95% of the cases. The SMN1 gene has been mapped to 5q13 region, which contains multiple copies of genes. There is a centromeric copy of the SMN gene, SMN2 whose copy number influences clinical severity.
The aim of this publication is to show the summary of the results of molecular diagnosis on spinal muscular atrophy which has been performed since 1993 exclusively by our laboratory in Hungary.
For the research of the genetic background influencing the phenotypes, homozygous deletions in exon 7 and 8 of SMN1 and in exon 5 of NAIPt genes were recorded and correlated with the type of clinical severity in 147 SMA families. Lack of SMN2 gene was also detected and the ratio of homozygous deletions in the parents of the patients in different clinical subgroups was calculated.
According to our data deletion of exon 5 of NAIPt occurred more frequently in SMAI (55%) than in SMAII (16%) and SMAIII (3.5%) patients. Homozygous deletions of the SMN2 gene in parents were only found in the SMA I group.
Our results correspond well to the data published by other researchers and shows that the size of the deletion in 5q13 might have an influence on the phenotype of the disease.

P0609. Identification of mutation in family of slovak Roms with Crigler-Najjar syndrome type I

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Crigler-Najjar syndrome type (CN I) is a rare autosomal recessive disorder, which is characterized by nonhemolytic severe unconjugated hyperbilirubinemia. The disease is due to hepatic dysfunction of uridine-diphosphoglucuronosyltransferase activity (UGT, E.C. 2.4.1.17) toward bilirubin. UGTs are a family of enzymes that conjugate various endogenous and exogenous compounds with glucuronic acid and facilitate their excretion in the bile. Bilirubin UGT is the only isoform that significantly contributes to the conjugation of bilirubin. Complete inactivation of this enzyme causing Crigler-Najjar syndrome type I leads to accumulation of unconjugated bilirubin in serum and bile, usually above 340 µmol/l level, which may cause later bilirubin encephalopathy (kernicterus) and death in infancy or childhood. Here we report the results of the molecular characterization of the UDP-glucuronosyltransferase gene (*UGT1A1*) in a family of Slovak Roms with CN I. Out of 12 children, born to consanguineous parents, we have examined 4 patients with CN I and their 3 unaffected sibs, aged 4 to 11 years. Primers we used were self designed. Sequence analysis of both the specific exon 1 of *UGT1A1* gene and common exons 2-5 in all 4 patients showed mutation in exon 4, the previously described deletion of an A at codon 407 (delA1220). Deletion A at codon 407 results in a premature stop codon and until now wasn't described in homozygous status. All of our patients were homozygous for delA1220 mutation and another 3 sibs were heterozygous. The homozygosity of this mutation in our patients is a consequence of consanguinity among the parents.

P0610. Molecular detection of the calpain defect causing limb-girdle muscular dystrophy type 2A in a Hungarian family

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Limb girdle muscular dystrophies (LGMD2) are a clinically and genetically heterogeneous group of autosomal recessive diseases, characterized by progressive atrophy and weakness predominantly in the proximal limb muscles. We present our clinical, histological, immunohistochemical, immunoblot and genetic results of two sisters suffering from so far unclassified autosomal recessive limb girdle muscular dystrophy. Haplotype analysis for genes known to be involved in autosomal recessive limb girdle muscular dystrophy was performed in the genetically informative family. On Western blotting the specific bands of calpain3 were absent while with the actin antibody normal band intensity was present. This suggested a primary defect of the calpain protein. Direct sequencing the 24 exons revealed that the two siblings have the same compound heterozygous mutation: in exon 4 there was a 1bp size deletion (c.550delA) while a missense mutation Arg355Trp (CGG>TGG; c.1063C>T) was found in exon 8. Both of these mutations were already published in the LGMD2A database (http://www.dmd.nl/capn3_home.html), therefore their pathogenic role is highly likely. The c.550delA mutation is relative frequent in the European population. In the Russian ethnic group this mutation has been reported to be conserved with a founder haplotype. It can be assumed that this mutation might be frequent in the Hungarian LGMD2A patients as well.
We would like to emphasize the importance of the above described combined strategy in diagnosing limb girdle muscular dystrophies

P0611. The JAG1 gene mutations in Polish patients with Alagille syndrome

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Alagille syndrome (AGS) is a multisystem, autosomal dominant disorder characterized by abnormal development of the liver, heart, skeleton, eye and face. Many studies have demonstrated that mutations in the *JAG1* gene result in the AGS phenotype. The *JAG1* gene encodes a transmembrane protein which is a ligand in the evolutionarily conserved Notch signaling pathway, taking part in cell fate determination and differentiation. In this study a group of 31 Polish patients with clinically recognized Alagille syndrome and their families were investigated to estimate frequency and type of mutation in the *JAG1* gene. Sixteen different mutations, including 12 novel and 4 recurrent changes, were identified. Among them are 7 frameshift, 5 nonsense, 2 splice site and 2 missense mutations. Most of the mutations (75 %) are suspected to result in truncation of the JAG1 protein. Forty percent of the changes were identified in exons 2 and 4, coding a highly conserved region. The remaining mutations are spread along the entire coding sequence of the gene. Screening of available parental samples revealed that in 3 out of 11 investigated families the specific mutations were inherited from mother (2 cases) or father (1 case). The low number of inherited mutations indicates that a significant number of AGS cases are sporadic or due to undetected parental mosaicism.
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P0612. *CFTR*dele2,3(21kb) in German patients

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The mutation *CFTR*dele2,3(21kb) is characterized by the loss of exons 2 and 3 in the *CFTR* mRNA. This results in premature termination of translation due to use of a premature stop codon within exon 4. In Germany, the *CFTR*dele2,3(21kb) was detected in 1.5% of all cystic fibrosis (CF) chromosomes (Dörk et al., 2000) suggesting that it should be included in routine CF diagnosis.
1390 DNA samples of affected patients, relatives and persons at risk were tested retrospectively for the presence of the *CFTR*dele2,3(21kb) mutation. We detected seven mutated alleles.

Three were found in a compound heterozygous state of severely affected patients and four alleles in healthy carriers. All affected children carry the *CFTR*dele2,3(21kb)/dF508 genotype. Furthermore, we deduced the genotype of a deceased girl with the diagnosis of „atypical CF“. No DNA probe of the girl was available but her parents were tested positive for mutations *CFTR*dele2,3(21kb) and R347P, respectively. Pedigrees and clinical information of affected patients will be presented.

P0613. A re-classified and a new ICF patient suffer from DNA hypomethylation

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The ICF sydrome (OMIM#242860) is a rare autosomal recessively inherited disorder. At a young age, patients suffer from recurrent infections, primarily of the respiratory system, due to an immune deficiency. Chromosomal centromeric instability of chromosome 1, 9 and 16 is a hallmark feature of the syndrome, resulting frequently in radial configurations. Commonly, patients display facial anomalies and mental retardation of varying degree. Most ICF patients carry mutations in the gene for the DNA methytransferase 3b (DNMT3b) and consequently hypomethylation of satellite II DNA is observed. Another syndrome (OMIM#243340) in a boy from consangineous parents also displays recurrent infections of the respiratory system, facial anomalies and mental retardation, features that resemble those of the ICF sydrome. Additionally, polydactyly, hypoplasia of the ischiadic bones, and complex renal dysfunction were also diagnosed. Due to the similarity of some aspects of both syndromes we asked whether they share underlying molecular defects. We analysed this patient as well as a new ICF patient at the molecular and cytogenetic level. The ICF hallmark features, namely radial chromosomal configurations and satellite II hypomethylation were found in both patients. The DNMT3b sequence of the putative promoter and coding region is normal. Aberrant alternative splicing was not observed. From our data we conclude that both syndromes may result from deficient DNA methylation, possibly due to second site mutations.

P0614. Identification of a new mutation (G56D) in the arginine vasopressin receptor 2 gene (*AVPR2*) in a familiy with X-linked nephrogenic diabetes insipidus

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Congenital nephrogenic diabetes insipidus (NDI) is characterized by polyuria and polydipsia due to an inability to concentrate urine despite normal or elevated plasma concentrations of the antidiuretic hormone arginine vasopressin (AVP). In 90% of families the disease (MIM304800) is inherited in a X-linked recessive manner, caused by mutations in the *AVPR2*-gene on Xq28, mostly in or near regions coding for the transmembrane domains of the receptor. Molecular genetic testing of the *AVPR2* gene detects more than 97% of disease-causing mutations in individuals with X-linked NDI. Identification of mutations in the *AVPR2* gene can facilitate early diagnosis of NDI, thereby preventing serious complications such as growth and mental retardation. This study describes the presence of a new potentially disease-causing point mutation, 701G>A, resulting in the amino acid substitution G56D, in the first transmembrane domain of *AVPR2* in an affected male. Family analysis revealed the mutation in the patient's unaffected mother, his mildly affected grandmother and in two unaffected female relatives. His unaffected brother carried a normal *AVPR2* allele. Documentation of the diversity of mutations will assist in revealing the full spectrum of clinical variation and may contribute to early diagnosis and treatment of NDI.

P0615. Transient neonatal diabetes mellitus (TNDM) :molecular diagnosis using methylation sensitive PCR

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Transient Neonatal Diabetes Mellitus (TNDM) is a rare form of diabetes occurring in about 1/400,000 births. It presents in growth retarded neonates within 6 weeks of birth with persistent hyperglycaemia. Patients usually require exogenous insulin therapy but the condition is transient with recovery by 18 months of age. However about 40% of patients relapse and develop type 2 diabetes later in life. A large proportion of TNDM cases are caused by three abnormalities of chromosome 6 involving an imprinted locus at 6q24. In our cohort of 63 patients we have identified, using standard methodology, 17 (27%) patients with paternal UPD of chromosome 6, 21 (33%) patients with paternal duplication of the 6q24 TNDM critical region and 11(17%) patients with undermethylation of the maternally inherited homologue of an imprinted CpG island at 6q24. In a further 14 (22%) cases no abnormality of chromosome 6 has been identified. We have now developed a method based on bisulphite treated DNA and methylation sensitive PCR which can rapidly identify all three abnormalities of 6q24. This method has correctly identified the nature of the mutation in 38 samples with 6q24 abnormalities. There were no false positives or false negative results. The samples consisted of 10 with paternal UPD6, 6 with a methylation defect at 6q24, 15 with a paternal duplication including 6q24, 1 sample with maternal UPD6 and 6 with a maternal duplication including 6q24. 14 normal control samples were also correctly assigned.

P0616. Molecular analysis of human and mouse Pax6 homeobox missense mutants.

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Mutations of Pax6 gene cause aniridia in humans and the Small eye phenotype in mice. Pax6 binds DNA through two conserved domains: the Paired domain (PRD) and the Homeodomain (HD). In contrast to the PRD, very few missense mutations have been found in Pax6 HD in humans or mice, and none of these was studied in terms of DNA binding. In this investigation, we have studied the DNA-binding properties of the three missense HD mutations found so far in humans and mice: R242T, (human); V256E and S259P (mouse). The R242T mutation was described in a child with unilateral iris coloboma and normal visual acuity. The V256E phenotype is very similar to the Small eye, while S259P has a milder phenotype.
The binding properties of these mutants were compared by gel-retardation assays to those of the wild-type Pax6 HD. The experiments were performed using either isolated HDs or PRD-HDs, with different oligonucleotides. The R242T mutant shows the same binding activity of the wild-type HD, while for the two V256E and S259P mutants the DNA binding activity is abolished. Since mutant R242T does not show a DNA-binding defect, its transactivation activity was investigated by cotransfection assays. HeLa cells were transfected with reporter plasmids containing Pax6 binding sites and either the R242T mutant or wild-type expression plasmids. R242T mutant shows a transcriptional activity three-four folds higher than the wild-type protein. These data indicate that developmental defects due to Pax6 HD missense mutations may be due to molecular mechanisms independent from the DNA-binding function.

P0617. PCR detection of Y specific amelogenin sequence in Turner Syndrome by modified primer set

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Y chromosome material is detected in up to 6% of patients by karyotype analysis, by molecular approach this frequency varied from 0% to 61% depending on the molecular methodology used. A nested PCR was considered a non reliable method by Nishi et al., 2002 because of the risk of contamination that overestimates the frequency of Y specific sequences.
We have developed a high sensitivity methodology based on Y specific amelogenin sequence amplification. The amelogenin sex test is widely screened in several populations, but the major drawback

is the competition that can occur when a small amount of male DNA can be inhibited by a large excess of female DNA. To overcome these difficulties we have design a new primers set using nucleotides differences in the X and Y homologous region, choosing mismatches at 3' end between Y and X sequences. We tested this approach amplifying samples with males DNA with known concentration diluted 1:1000, 1:10000 and 1:100000 in females excess. This new primers successfully amplify only Y region even with a 100.000 fold excess of female DNA. As a control the 6-bp insertion in amel Y is included in the amplicons.
We tested 41 patients with Turner syndrome via modified primer set and Y specific sequence was found in 4 cases supported by karyotype analysis and in 2 without cytogenetic evidences.
We conclude that amelogenin amplification with modified primers is a useful tool to detect cryptic Y chromosome material showing high sensitivity and minimal risk of contamination.

P0618. Molecular genetics of G6PD and determination of two kinds of mutations in G6PD gene in a family in state of Golestan

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Glucose -6- Phosphate Dehydrogenase is a highly polymorphic enzyme that is encoded by X-link (Xq28) human gene. G6PD deficiency is the most common human metabolic inborn error, which affected more than 400 million people worldwide. The main clinical manifestations are neonatal jaundice and acute hemolytic anemia. Up to now, more than 100 molecular variants have been identified. In the present study we have detected three common molecular variants of G6PD analyzed on peripheral blood samples of 71 patients with G6PD deficiency in state of Golestan in the north of Iran by RFLP technique. In this ways, it revealed that Mediterranean mutation is predominant in this area 69% and 26.8% have Chatham mutation, but none of the samples were found to be Cosenza mutation. In this study, we also faced on an interesting case; a family with five members including parents, two sons and one daughter, while two of them (father and the youngest son) have normal G6PD activity and three of them (mother, the eldest son and daughter) are G6PD deficiency. The eldest child is an affected boy in hematology and molecular genetic tests. Molecular analysis showed Chatham mutation in him. Their daughter has acute anemia symptom too. Molecular analysis showed her heterozygous in both of Chatham and Mediterranean mutations. While the mother showed weak symptoms about G6PD deficiency with Chatham mutation. The details of this study will be discussed.

P0619. Neural stem cell proliferation and differentiation in the absence of cathepsins B and L

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Progressive myoclonus epilepsy of the Unverricht-Lundborg type (EPM1) is caused by loss-of-function mutations in the cysteine protease inhibitor cystatin B. Cystatin B knockout mice reproduce many EPM1 features which can be partially rescued by removal of the cysteine protease cathepsin B. This suggests that cathepsin B contributes to EPM1 pathogenesis when it is not regulated by its physiological inhibitor cystatin B. Moreover, the identification of cathepsin B substrates will likely add to elucidate the molecular basis of EPM1. We have previously shown that cathepsins B and L functionally compensate for each other *in vivo*. Mice deficient for both cathepsins show a dramatic accumulation of lysosomes within selective brain neurons. We here confirm subsequent apoptosis of cathepsin $B^{-/-}L^{-/-}$ forebrain neurons by immunohistochemistry with an antibody against activated caspase-3 and by electron microscopy. To further analyze cell-type specific effects of cathepsins B and L, we isolated and expanded neural stem and progenitor cells from 12-day-old fetal forebrains of wild-type, cathepsin $B^{-/-}$, and cathepsin $B^{-/-}L^{-/-}$ mice. Their proliferative potential is quantified based on their capacity to generate primary and secondary neurospheres in culture. Furthermore, neurospheres are tested for their ability to differentiate into intact neurons, astrocytes, and oligodendrocytes. In addition to investigating the effects of cathepsins B and L on proliferation and multilineage potential of neural stem and progenitor cells, the suitability of cathepsin $B^{-/-}L^{-/-}$ stem cells for proteomic approaches is evaluated with the aim to identify neuron-specific *in vivo* substrates for cathepsins B and L.

P0620. Detection of mutations in *post-morten* well characterized samples in familial Alzheimer disease

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In early-onset familial Alzheimer's disease (AD) pathogenic mutations have been found in the amyloid precursor protein (APP) gene, and the presenilin (PS)-1 and PS-2 genes. We screened for mutations in these genes in 6 patients with familial AD from the Spanish population. These patients have confirmed AD and one amyloid angyopathy only. We detected one pathogenic change in one familial patient (M139T), one polymorphism change in one familial patient (E318G) and one not well characterized change in the APP gene (A713T). The 713 codon has been related with schizophrenia, and the change A713T has been described as a polymorphism. We have not found this change in the APP gene in control population, and we concluded that this polymorphism could likely be related to AD.

P0621. Molecular diagnostics in genetics : techniques and difficulties

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Although the human genome project is nearly completed, and all human genes are known, molecular diagnostics for genetic diseases remains a challenging problem.
Whereas the disease gene for more than 1000 genetic disorders has been identified, molecular diagnostics are available for only a minority of these in most countries. Furthermore, the analysis is often incomplete, slow and expensive. The most important bottlenecks for cost-effective molecular diagnostics are the nature of the mutation and the rareness of genetic diseases in general.
Some disorders caused by a single or a few mutations can be easily diagnosed by molecular methods, but the vast majority of genetic diseases are caused by private mutations specific for a single family . Consequently, the whole gene has to be analysed by complete sequencing, or screening methods such as WAVE, DGGE or SSCP followed be sequencing of the abnormal fragments. This laborious methodology is only cost-effective when enough samples are available. Due to the rareness of most of the genetic disorders this can not be set up cost-effectively on a regional or even national level.To facilitate genetic diagnostics an international network of diagnostic labs was organised, existing of many referral labs worldwide, 20 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 diagnostic tests for more than 400 genetic diseases (www.GENDIA.net).

P0622. Mutational analysis of xanthine dehydrogenase gene in four Japanese patients with classical xanthinuria type I.

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Classical xanthinuria is an inherited metabolic disorder caused by a deficiency of xanthine dehydrogenase (XDH), resulting in hypouricemia. Classical xanthinuria frequently present with renal calculi and occasionally lead to renal failure. Classical xanthinuria is classified into two categories. Classical xanthinuria type I lacks only XDH activity by XDH gene mutations, while type II results from dual deficiency of XDH and aldehyde oxidase due to mutations in the molybdenum cofactor sulfurase gene. Classical xanthinuria is easily differentiated by means of the allopurinol loading test. As allopurinol

is converted to oxypurinol by both XDH and aldehyde oxidase, oxypurinol is detected in urine and serum of patients with classical xanthinuria type I upon administration of allopurinol. We have identified XDH gene mutations of new four xanthinuric Japanese who have no kinship each other. The results of allopurinol loading test for two patients indicated xanthinuria type I. For the other two patients, we diagnosed their xanthinuric type by XDH gene analysis. Urolithiasis was not found in any patient. Urolithiasis frequency in xanthinuric patients including our cases in Japan has been less than in other countries. It is probably related with water quality, climate and food. We identified homozygous novel point mutations C3853T (Q1285Ter) and G446A (R149H) and homozygous deletion C2567Del in two patients. The amino acid sequences around R149H were well conserved in most species. These findings indicate that this region will be important for maintaining the XDH activity.

P0623. Search for mutations in Russian patients with hypertrophic cardiomyopathy

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To study mutations causing hypertrophic cardiomyopathy (HCM) in Russia, consecutive HCM patients (N=25) were recruited at the Tomsk Cardiological Centre. Clinical examinations were based on standard criteria including exercise tolerance tests, echocardiography and blood pressure monitoring (to exclude systemic hypertension). Three cases were clearly familial, whereas others were presumably sporadic. Two major HCM genes were investigated by SSCP analysis and subsequent DNA sequencing, *MBPC3* and *MYH7*. *MBPC3* was studied in 25 individuals, and *MYH7* was investigated in 11 individuals. Three mutations were identified in *MYBPC3* and three in *MYH7*. The cause in one family was resolved, in two others not. Five mutations were found in sporadic patients. Four of the mutations were new (Leu714Arg and Arg1712Trp in *MYH7*, int16 +1g→a and int22 +2t→g in *MYBPC3*). Two were described before (Arg663His in *MYH7* and Gln1233ter in *MYBPC3*). These mutations were not detected in a population control (N>100). Mutations in *MYBPC3* are predicted to affect splicing or to cause truncation of the protein. The changes in MYH7 are missense mutations. The clinical manifestation of the Leu714Arg mutation was unusually severe in two identified carriers (father and son). This is the first systematic study of HCM causes in a Russian population. Further investigations will be focussed on relatives of presumed sporadic cases. The fact, that four of the mutations were new, extends the range of the known genetic heterogeneity of HCM causes. Current evidence suggests variable severity (or penetrance) of the mutations identified by us.

P0624. Diagnosis of haploid and triploid based on measurement of gene copy number in CMT and HNPP.

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A 1.5-Mb tandem duplication, including the PMP22 gene in chromosome 17p11.2-12 is responsible for 70% of the cases of Charcot-Marie-Tooth disease (CMT1A). A reciprocal deletion of this region causes the hereditary neuropathy with liability to pressure palsies (HNPP). We report the results obtained by Real Time PCR in 25 HNPP, 71 CMT and 58 normal controls based on measurement of gene copy number. The CMT1A unrelated patients were previously analyzed by the presence of 3.2 Kb junctional fragment and microsatellite markers. 43/71 (60.56%) revealed the 3.2 fragment, 63/72 (85,91%) could be identified by combined use of PCR and microsatellite markers . The duplication in the samples by microsatellite analysis was inferred when at least one of the seven microsatellites showed the presence of three different alleles. 10/71 (14,09%) of the patients did not show either 3.2 band or three different alleles, but they showed an allelic ratio indicative for a duplication. In order to confirm the duplication in this group, we performed PFGE that revealed the duplication. By these methods were also analyzed the HNPP patients.

By Real Time PCR in the normal controls a ΔΔCt ratio range from 0.788 to 1.164 (0.98±2(0.094)) has been identified. All patients with the duplication showed values higher than 1.164, whereas the HNPP patients revealed values below 0.72. The results indicate that Real Time PCR shows superior sensitivity to microsatellite analysis and has the additional advantage of being a fast and uniform assay for quantitative analysis of both CMT1A and HNPP.

P0625. Genotype- Phenotype correlations for a wide spectrum of mutations in the ATP7B Gene

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Molecular analysis of the ATP7B gene on 93 Greek Wilson disease (WND) index patients from 69 unrelated families and their relatives resulted in the characterization of twenty different mutations accounting for 86.13% of the WND chromosomes. The most frequent were: H1069Q (34.78%), R969Q (12.31%), 2530delA (7.24%), L936X (6.52%), Q289X (6.52%), and I1148T (2.89%), while 81.15% of the mutations are concentrated in 8 exons. Thirty cases proved homozygous for 9 different mutations, 22 of which were index cases. Patients that are homozygous for H1069Q and R969Q have a milder disease presenting at an older age. These two missense mutations ameliorate the disease severity even in compound heterozygosity. At the other end of the disease spectrum are patients carrying protein-truncating mutations (nonsense and frameshift), that lead to more severe disease with a significantly earlier age of onset and lower ceruloplasmin level (P 0.004 for both). Additionally a significant difference was found in the serum copper value (P 0.05) for mutation 2530delA. Clinical phenotype is associated to some extent with the disease-causing mutations but other genetic factors and environmental influences could also have a direct effect on the severity of the disease symptoms which would explain the marked clinical heterogeneity that we observed, even within sibs sharing the same genotype. An early diagnosis of WND preferably at the presymptomatic stage, prevents disabling residual symptoms. Therefore knowledge of the disease mutation spectrum for a specific population allows more efficient mutation detection.

P0626. Detection of deletions in exons 7 and 8 of the SMN gene in SMA patients in Khouzestan province from Iran

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The term SMA is used for a clinically and genetically heterogeneous group of neuromuscular disorders.Two types of disease occur prenatally which include AMC and CAN. Childhood SMAs are divided into types I, II and III.Patients are distinguished on the basis of age of onset and severity of the clinical course as assessed by clinical examination, muscle biopsy and electromyography.

Four genes SMN, NAIP, P44, and H4F5 have been mapped to a 850kb interval on 5q13, which is involved in the causation and severity of SMA. The SMN gene exists as a telomeric copy (tel SMN) and a highly homologous centromeric copy (cen SMN). Both copies show identical sequences, except for five single nucleotide changes at the 3'- end of the gene (intron 6 to exon 8). However only deletion / mutation in tel-SMN is seen to cause SMA. Homozygous deletions of cen-SMN were found in about 2-3% of carriers and controls. In this research, we have studied deletions of exon 7 and 8 of the SMN gene using PCR/RFLP techniques. 33 individuals from 25 families in Khouzestan were investigated for the SMN gene deletion. Genetic counseling and pedigree analysis were performed for indivduals. 88% of cases were positive for presence of the above deletions. Using the procedure described here we will be able to detect the carrier individuals.

P0627. A new locus for a childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia maps to chromosome 11p15

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The cerebellar ataxia's are a heterogeneous group of neurodegenerative disorders, characterised by symptoms and signs of cerebellar degeneration and by pyramidal and extrapyramidal features as well as polyneuropathy in a variable extent. The clinical picture shows large variation in age at onset and disease progression. The mode of inheritance in hereditary ataxia's can be autosomal dominant, autosomal recessive or X-linked.
Here we report a non-consanguineous Dutch family with a pure spinocerebellar phenotype with cerebellar ataxia, pyramidal signs, posterior column involvement with deep sensory loss, a postural tremor and absence of other (non-) neurological features. Neuroimaging shows atrophy of the cerebellum, vermis, pons and medulla oblongata. Onset of symptoms is in early childhood but remarkably, the severity of symptoms and progression of the disease within this family is very variable. The clinical phenotype of the family is not consistent with any of the known autosomal recessive cerebellar ataxia's.
Using a systematic genome wide scan we mapped the responsible gene for autosomal recessive ataxia in this family to a 5.9 cM interval on chromosome 11p15. A large number of genes and expressed sequence tags are identified in this critical region. No obvious candidate gene can be assigned, as genes for ataxia mostly have different functions and features.

P0628. Difference in allelic expression of the *CLCN1* gene and the possible influence on the myotonia congenita phenotype

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Mutations in the *CLCN1* gene, encoding a muscle-specific chloride channel, can cause either recessive or dominant myotonia congenita (MC). The recessive form, Becker's myotonia, is believed to be caused by two loss-of-function mutations, whereas the dominant form, Thomsen's myotonia, is assumed to be a consequence of a dominant negative effect. However, a subset of *CLCN1* mutations can cause both recessive and dominant MC. We have identified two recessive and two dominant MC families segregating the common R894X mutation. Real-time quantitative RT-PCR did not reveal any obvious association between the total *CLCN1* mRNA level in muscle and the mode of inheritance, but the dominant family with the most severe phenotype expressed twice the expected amount of the R894X mRNA allele. Thus, the *CLCN1* gene is subject to variation in allelic expression. Variation in allelic expression has not previously been described for *CLCN1*, and our finding suggest that allelic variation maybe an important modifier of disease progression in myotonia congenita.

P0629. A mosaic pattern of common recurrent and founder DHCR7 mutations causing the Smith-Lemli-Opitz Syndrome in Europeans

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Mutations in the DHCR7 gene cause the Smith-Lemli-Opitz syndrome (MIM 270400), an autosomal recessive metabolic malformation and mental retardation syndrome occurring predominantly in Caucasians. Over 80 different mutations have been detected, some of which are frequent. Mutational spectra in SLOS patients vary significantly across Europe (Witsch-Baumgartner et al., 2001).
Here we have analysed haplotypes associated with IVS8-1G>C, T93M, R404C, and G410S using intragenic SNPs in unrelated SLOS patients originating from France, Germany, Greece, Ireland, Italy, Spain, Turkey, and the United Kingdom. For the T93M mutation 6 different haplotypes A, B, C, F, J, and P were observed. The R404C mutation was found associated with 4 different haplotypes A, B, D, and F and the G410S mutation with two haplotypes A and F. All three mutations were associated with specific haplotypes depending on the geographic origin of the SLOS patient, suggesting recurrent founder origins for these mutations. The data indicate that T93M founder mutations have occurred in the Mediterranean region. Similarly the R404C mutation has founders in France, UK, and in Germany. The G410S mutation has appeared twice, once in the Eastern Mediterranean region and once in Western Europe. In contrast the splice site mutation IVS8-1G>C was found associated with only one haplotype (A) indicating one historically old founder effect the age of which was calculated at 3000 years using flanking microsatellites. Together this suggests that DHCR7 mutations have independently reached high frequencies in different European populations indicating selection.

P0630. Mutation detection of CFTR gene using ARMS and SSCP methods in Iran

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Cystic fibrosis (CF), the most common severe lethal autosomal recessive disorder in whites, is caused by mutation in the CFTR gene on chromosome 7q31. The carrier frequency among Caucasians is approximately 1 in 25, with an incidence of approximately 1 in 2500 live birth. CF patients have two defective alleles and may either be heterozygous for different mutations, or homozygous for one of the mutations.
Since the identification of the gene responsible for CF, more than 1000 mutations was described in CFTR gene of patients affected by cystic fibrosis, but the prevalence of the CF shows a geographical and ethnical variations in the world. The Delta F508 mutation in CFTR gene accounts for over 70 % all mutant CFTR alleles in the Europeans to 20 % in the Asians.
First the DNA samples of 92 CF chromosomes has been tested for eight common mutations: ΔF508, W1282X, 621+1G>T,1717-1G>A, G542X, G551D, N1303K, R560T by using the ARMS method .
Results from this study revealed the following frequencies: ΔF508: 19.5%, G542X: 10%, W1282X: 6.5%, N1303K: 1.1%, 621+1G>T: 0%,1717-1G>A: 0%, G551D: 0% , R560T 0%.Then by using SSCP method for samples which we didn't find any mutation by ARMS system, we scanned exons 4, 7, 10, 11 and 13 of CFTR gene. By using sequencing we find two mutations in exon 4, one mutation in exon 7 and two mutations in exon 13.

P0631. Disease causing ARX gene mutation in the XLMR pedigree MRX33

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X-linked gene defects may account for one third of all idiopathic forms of mental retardation in males, and most of these cases are non-syndromic. The finding of causative mutations in 12 different genes as well as linkage studies have established that non-syndromic

XLMR is highly heterogenous; until recently it was believed that up to 100 X-linked genes might be involved. However, as judged from the recent findings that up to 20 percent of all patients with this condition have mutations in the ARX gene, the total number of genes may be much lower. We have previously reported a large family (MRX33) with non-syndromic XLMR and showed that the genetic interval containing the disease-related genes maps to Xp11.4 – Xp22.12. As homeodomain genes are known to play crucial roles in the development of cerebral structures, ARX was considered as a strong candidate gene for this XLMR family, as it has been mapped to this region. To search for mutations in the MRX33-family, we used direct sequencing of ARX-PCR products. The analysis revealed the presence of a 24bp duplication in exon 2 duplicating nucleotides 428-451. This resulted in a polyalanine expansion from a tract of 12 alanines (amino acids 144-155) to a tract of 20 alanines. This duplication is known to be causative for XLMR in 3 families and for the Partington syndrome in 2 families. Here we describe the fourth XLMR family with a mutation in the ARX gene.

P0632. Genotype-phenotype correlation and founder effect in SMA linked to 5q13 without homozygous deletion of SMN1

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Spinal muscular atrophy (SMA) is a common autosomal recessive disease linked to 5q13 locus in 95% patients. SMN1 homozygous deletion is found at least in 98% of them. In 1.8-3.8%, a genotype associating SMN1 deletion and a subtle mutation was reported. Using long range PCR and sequencing, we found 10 mutations including five mutations never reported so far and five recurrent mutations. Overall reported families carrying a subtle mutation is 77: 69% showed a mutation in amplicon 6 (45.5%) or 3 (23.4%). The most frequent mutations described in different populations are c.815A>G (13%) and c.770_780dup11 (11.7 %) in exon 6. Both showed common haplotypes in all patients with the same mutation, supporting the hypothesis of a founder effect rather than mutation hot spot. Other mutations are probably population specific as 439_443delTGAAG in Italy, c.399_402delAGAG in Spain or c.585_586insT and c.885+3del4 in France.
Moreover, SMN2 dosage in 19 heterozygous compounded SMA patients correlates with the phenotype, except for exon 5 and 6 deletion showing 3 SMN2 associated to a severe phenotype (SMA I). This deletion could have a toxic effect. As exon 6 contains a self oligomerisation domain, this deletion can generated an abnormal dimerisation of the Smn protein. In SMA, other molecular mechanisms than haploinsufficiency are possible.

P0633. Novel mutations in the gene SALL4 provide further evidence for Acro-Renal-Ocular and Okihiro syndromes being allelic entities, and extend the phenotypic spectrum.

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Okihiro syndrome results from mutation in the putative zinc finger transcription factor gene SALL4 on chromosome 20q13.13-13.2. To date, 13 different mutations have been reported. These mutations have been found in patients with Okihiro syndrome as well as in patients who received the primary diagnosis of Holt-Oram syndrome, acro-renal-ocular syndrome, and Thalidomide embryopathy. However, patients diagnosed with acro-renal-ocular syndrome show markedly different involvement of the eye, ranging from Duane anomaly to structural eye defects. In order to extend the genotype-phenotype correlations in Okihiro and related syndromes, we performed further mutation analysis in patients with those conditions. Here we report five novel SALL4 mutations in five unrelated families, among them a family previously reported as acro-renal-ocular syndrome with the affected members showing structural eye defects but no Duane anomaly. Another interesting observation is that SALL4 mutations can also be associated with severe heart defects i.e. tetralogy of Fallot. Our results therefore extend the phenotypic spectrum of SALL4 mutations and provide further proof that Okihiro and acro-renal-ocular syndromes are allelic conditions.

P0634. Inherited defects in structural components of the cartilage extracellular matrix of chondrodysplasias

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The human chondrodysplasias are a heterogeneous group of heritable connective tissue disorders, which are caused by defects in endochondral ossification.
A difference in the lectin binding patterns of normal and diseased growth plates has been established (Horton 1982, Miosge et al 1998). Abnormal tissues showed alteration in carbohydrate residues compared to normal, even in those cases with the same histology as the normal growth plate. Lectins have been used in a variety of tissues to examine differentiation and maturation involving the sugar moieties of both cellular and matrix glycoconjugates and study distribution, concentration and organisation of matrix carbohydrates. .
In the present study the growth plates from the costochondral junctions were sub-divided into two groups: normal and pathological. A panel of 16 biotinylated lectins was applied to these two groups under the same conditions: HHA, PSA, LCA, e-PHA, l-PHA, UEA-I, AAA, ECA, CTA, AHA, DBA, VVA, SBA, HPA, WFA and DSA.
The results obtained showed dissimilarity in glycosylation patterns between the matrices of the normal and abnormal growth plate. In addition, a group of growth plates, known to be sites of secondary malformation accompanying the primary defect, although displaying a histologically normal appearance, also showed an altered glycosylation pattern. Skeletal disorders were all associated with a loss of bisection in N-glycans, strongly suggesting that GnTIII is defective and its gene is, therefore a target for further analysis. Variation between the disorders was consistent with variation in the point in the processing reactions at which GnTIII was acting.

P0635. Carnitine palmitoyltransferase II deficiency: Biochemical and mutation analysis in patients detected by tandem mass spectrometric acylcarnitine profiling

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Mitochondrial carnitine palmitoyltransferase II (CPT II) deficiency is the most common inherited disorder of lipid metabolism in adults. Patients suffer from recurrent myoglobinuria, muscular weakness and myalgia, triggered by exertional exercise, cold, infection, and/or prolonged fasting. We have previously shown that tandem mass spectrometric serum acylcarnitine profiling is a sensitive, rapid and non-invasive tool for detection of CPT II deficiency (Gempel et al., 2002). Seven newly diagnosed patients were further characterized by measurement of CPT II activity in leukocytes and/or muscle biopsy and by mutation analysis of the *CPT2* gene. The most sensitive (C16+C18:1/C2) acylcarnitine ratio was clearly elevated in all patients (range: 0,119-0,263; reference range: 0,011-0,048). CPT II activity in leukocytes as measured in the backward direction and inhibition of CPT I by 5 % Triton X-100 ranged from 0,01 to 0,46 nmoles/min/mg protein (reference range: 1,0-2,5). A combined PCR-RFLP and gene sequencing approach revealed six different pathogenic mutations which have been previously described in association with the muscular phenotype of CPT II deficiency. Two patients were homozygous for the common S113L mutation, while three patients had the S113L mutation in combination with E487K (two brothers) or 1238_1239delAG/F448L. The second-most prevalent mutation P50H was found in one patient together with the R124X mutation. The seventh patient was homozygous for the R161W mutation. In conclusion, we show that CPT II deficiency can be safely and rapidly diagnosed from a small blood sample by a combined tandem mass spectrometric, biochemical and genetic approach.

P0636. Mutation analysis of MECP2 gene in Taipei City

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Background: Rett syndrome(RTT) is a neurodevelopmental disorder caused by mutations in the methyl-CpG binding protein 2 gene(MECP2). In order to understand the extent of MECP2 mutation in Chinese population, we analyzed MECP2 on 20 patients with clinical suspicious of RTT and 100 patients with idiopathic mental retardation in Taipei City.
Methods: All patients enrolled were first excluded fragile x syndrome through FMR-1 analysis. The coding sequences of MECP2 gene were amplified by PCR. For patients of idiopathic mental retardation, the suspected PCR products were sequenced after screened by denaturing high performance liquid chromatography (DHPLC). For patients of high risk for MECP2 mutation (either x-linked mental retardation suggested from family history or RTT suspected by neurologist or psychiatrist), PCR products were directly examined by automatic sequencing.
Results: The analysis revealed 6 disease-causing mutation from 8 sporadic patients, including one male case. Among these 8 patients, only 5 belonged to typical RTT phenotype. These mutations included 4 missense mutations and two deletions. Three mutations were located in the methyl-CpG-binding domain and one in transcriptional repression domain, two in C-terminal segment. Two mothers were noted of mutation carrier after familial investigation. No mutation was identified among 19 cases suggested of XLMR.
Conclusion: In addition to typical RTT phenotype, patients with MECP2 mutation had different clinical presentation in Chinese population. Our study provided an efficient approach to discover the domains of MECP2 mutation. This analysis facilitate the diagnosis of RTT at the molecular level and provide insight into the molecular pathology of Rett syndrome.

P0637. A new allelic variant K695M in the MEFV gene in a Turkish family suffering from FMF

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Familial Mediterranean Fever (FMF) is a recessive disorder characterised by episodes of fever and neutrphil-mediated serozal inflammation. The gene, MEFV, responsible for this disease, comprises 10 exons and 781 codons. Twenty-nine mutations, mostly located in the exon 2 and especially in exon 10 of the MEFV gene, have been identified so far. Here, we describe a 9 year old male child with a family history of FMF who suffered from recurrent fever accompained by pains in the abdomen. During a five years period he experienced attacks about once every six months. He was threated by colchicine for four years. Genomic DNA, extracted from peripheral blood lymphocytes of the proband and his parents, were analysed by polymerase chain reaction (PCR), followed by cycle sequencing using ABI 310 Genotyper system. We detected homozygously a mutation, AAG—ATG(Lys—Met), in codon 695 in the exon 10 of the FMF gene, MEFV, that resulted in a substitution of methionine for arginine (K695M) in proband. His parents were carrier for this mutation heterozygously. In addition, both proband and his father were found to be carrier for R202Q, the of which is unclear in the clinical reflection of the FMF. However, proband's father has been clinically more affected than mother. We suggest that this mutation (K695M) is firstly found in a Turkish family and causes to FMF. Also, the association of R202Q with K695M or with mutations should be investigated in carriers and/or patients with FMF due to clinical differences.

P0638. Mutations of the CFTR gene in Turkish patients with congenital bilateral absence of the vas deferens

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Mutations of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) can cause congenital bilateral absence of the vas deferens (CBAVD) as a primarily genital form of cystic fibrosis. The spectrum and frequency of CFTR mutations in Turkish males with CBAVD is largely unknown. We investigated 51 Anatolian patients who had been diagnosed with CBAVD at the Hacettepe University, Ankara, for the presence of CFTR gene mutations by direct sequencing of the coding region and exon/intron boundaries. We identified 27 different mutations on 72.5% of the investigated alleles. Two-third of the patients harboured CFTR gene mutations on both chromosomes. Two predominant mutations, IVS8-5T and D1152H, accounted for more than one-third of the alleles. Five mutations were observed for the first time. With one exception, all identified patients harboured at least one mutation of the missense or splicing type. In conclusion, although classic cystic fibrosis is relatively rare in Turkey, CFTR mutations are responsible for the majority of CBAVD in Anatolian males.

P0639. CFTR gene mutations in patients with CFTR associated disorders

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This study was undertaken to test the possible involvement of CFTR gene mutations and polymorphisms in etiology of male infertility, chronic pancreatitis, disseminated bronchiectasis and chronic obstructive pulmonary disease in Serbian patients.
We analyzed 110 patients with CFTR associated disorders (21 infertile men, 39 patients with chronic pancreatitis, 31 patients with chronic obstructive pulmonary disease (COPD) and 19 patients with disseminated bronchiectasis). CFTR gene was screened for presence of mutations and polymorphisms using the combination of PCR and subsequent mutation detection methods (HA, PSM, SSCP, DGGE, sequencing). Several mutations were detected: F508del, R74W, R75Q, G126D, 711+3A/G, V920L, L997F and F1052V. In all four groups of patients common polymorphisms (1716G/A, 2377C/T, 2694T/G, 3417A/T, 4002A/G, 4029A/G, 4404C/T, 875+40A/G, 1001+11A/T, M470V, IVS6 (GATT)m) were also detected. The frequencies obtained for mutations and 5T allele at Tn polymorphic site are shown in the table.

clinical status	mutations	frequency	PI (statistical significance)	5T	frequency	PI
male infertility	7/62*	11.29%	<0.0001	5/62*	8.06%	0.142
chronic pan-creatitis	2/78*	2.56%	0.855	0/78*	0	-
disseminated bronchiec-tasis	3/38*	7.89%	0.118	0/38*	0	-
COPD	11/62*	17.74%	<0.0001	8/62*	12.90%	0.018

*number of screened chromosomes
These findings suggest that CFTR protein may be involved in etiology of obstructive azoospermia and chronic obstructive pulmonary disease, but not in chronic pancreatitis and disseminated bronchiectasis in Serbian patients. Further investigations on a larger cohort of patients are needed for confirmation of these results.

P0640. Deletions and duplications in the dystrophin gene: retrospective and prospective MLPA analyses show complex rearrangements and previously undetected deletions

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Background: Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are common X-chromosomal disorders frequently caused by genomic rearrangements. Since 1988 we have used multiplex PCR to screen for deletions in the dystrophin (*DMD*) gene.
Methods: Using the novel multiplex ligation-dependent probe amplification (MLPA) method for genomic quantification of all 79 *DMD* exons we performed a retrospective analysis on 92 mutation-negative male cases. Furthermore, 31 female individuals, two chorion

villi samples and 21 male patients were tested prospectively. MLPA reagents were obtained from MRC-Holland. The PCR products were quantified on an ABI 3100 sequencer.
Results: Previously undetected deletions were found in four reinvestigated cases (delEX10-11, delEX19, delEX38 and delEX61-63). In addition we found 11 previously unrecognised duplications dispersed over the whole gene, corresponding to a duplication rate of 12% among deletion-negative and 6% among all patients. Similar results were obtained in the prospective study. In one individual we found a complex rearrangement involving a duplication of two regions: dupEX45-48 and dupEX54-56. In another patient a duplication of exons 46-53 coincided with a deletion of exon 37.
Conclusion: We conclude that MLPA is a highly sensitive alternative to multiplex PCR. It can also be used to detect female carriers. Approximately 7% of all deletions had been previously overlooked by multiplex PCR.

P0641. Leprechaunism with unimpaired insulin-binding caused by novel mutations in the insulin receptor gene

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The human insulin receptor (INSR) is a heterotetramer composed of two insulin binding α subunits and two tyrosinase kinase β subunits. Homozygous or compound heterozygous mutations within the insulin binding domain lead to severe impairment of insulin-binding associated with Leprechaunism, a syndrome characterized by excessive hyperglycemia with hyperinsulinism and early mortality, pre- and postnatal growth retardation and distinct dysmorphism, while mutations with preserved insulin-binding capacity were demonstrated in other insulin resistance disorders.
We report on a 9 year old girl with characteristic Leprechaunism phenotype and hyperinsulinism with hyperglycemia (400mg/dl), but with prolonged survival. Unexpectedly, insulin-binding assays in her fibroblasts revealed no significant alterations. Nevertheless, sequence analysis of the INSR gene showed that the patient was compound heterozygous for 2 novel missense mutations C186F and R256C in exon 2 und 3 within the insulin-binding domain. These mutations segregate in the family according to autosomal recessive inheritance, were not present in 96 control samples and affect highly conserved amino acids. Bioinformatic analysis suggests that the loss of a disulphide bond caused by C186F mutation results in the destabilization of loop and that the R256C mutation increases the tendency to form an alternative (non-native) disulfide bonding pattern in INSR, which is both likely to disturb protein function.Thus we demonstrate, that mutations in the insulin binding α subunit of the INSR gene leading to Leprechaunism may not necessarily end up in impaired insulin-binding, but may also alter the INSR function. Insulin-binding is therefore not a valid parameter for predicting INSR mutations as proposed.

P0642. Prevalence of GJB2 and GJB6 mutations in non-syndromic hearing loss among the Italian population

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Mutations in the gene encoding connexin 26 (GJB2) have been described as a major cause of genetic non-syndromic hearing loss (NSHL). Recently, a deletion involving connexin 30 (GJB6) was associated with autosomal recessive NSHL, frequently observed in patients from Spain, France and Israel. In order to evaluate the prevalence of GJB2 and GJB6 mutations in Italy, we have studied 69 patients with mild to profound NSHL or deafness, recessive or apparently sporadic. In addition, we have screened 285 control subjects with normal hearing to evaluate the carrier frequency. A mutation screening by DHPLC and sequencing of GJB2 gene and the detection by multiplex PCR of 342-kb deletion in the GJB6 were performed.
Among the patients, 20 (29%) showed molecular variants of GJB2. In addition to known mutations (35delG, delE120,V27I, R32H, V37I, R127H), a novel G109W mutation was found in a compound heterozygote with the 35delG. Four novel sequence variants (IVS1-6T>C, IVS1-2A>C, Y158Y, K221N) were also detected.
30 (11%) controls showed molecular variants of GJB2, 14 of which (carrier rate of 5%) having a disease-causing mutation (M34T, 35delG, R127H, E47X, 167delT). Four novel sequence variants (IVS1-6T>C, IVS1-2A>C, A/G at -8, D159N) were also detected.
Neither patients nor controls were carrying the 342-kb deletion in the GJB6, indicating that the occurence of this defect is restricted to certain populations.
In conclusion, GJB2 mutations resulted responsible for almost one third of the NSHL in our patients and the carrier frequency in the Italian population resulted very high.

P0643. Dilated cardiomyopathy and novel mutations in *ALMS1* in Alström Syndrome.

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Alström Syndrome is a rare progressive autosomal recessive disease. In the first decade of life it commonly presents as a severe cone-rod dystrophy, truncal obesity, acanthosis nigricans and progresses to include sensory neural deafness, male hypogonadism, and NIDDM in the second decade of life. The lifespan of affected individuals is shortened, with death usually occurring from renal or heart failure in the fifth decade. One more rarely reported clinical feature of Alströms is dilated cardiomyopathy, manifesting either within the first year of life when it often spontaneously regresses, or in the second decade.
We studied 7 families in which the index case presented with a dilated cardiomyopathy prior to the diagnosis of Alstrom syndrome being made. The gene for Alström syndrome, *ALSM1*, has been identified and thirteen protein truncating mutations have been published to date of which 6 are clustered in exon 16. Of these mutations one is associated with a dilated cardiomyopathy. We have identified seven novel *ALMS1* mutations in our Alström Syndrome families. The mutations are all protein truncating and 3/7 are located in exon 16.
There is no distinct clustering or distribution pattern of the *ALMS1* mutations associated with dilated cardiomyopathy compared to those previously reported. We therefore suggest dilated cardiomyopathy may be a more common feature of Alström Syndrome than previously recognised. A clinical screening service for *ALMS1* mutations has been established in Leeds.

P0644. Detection and estimation of heteroplasmy for mitochondrial mutations using Nanochip and Pyrosequencing Technology

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Disease causing mutations in mitochondrial DNA are typically heteroplasmic and therefore interpretation of genetic tests for mitochondrial disorders is problematic. The reliable measurement of heteroplasmy in different tissues may help identify individuals who are at risk of developing specific complications and allow improved prognostic advice for patients and family members. We evaluated the NanoChip® Molecular Biology Workstation and Pyrosequencing™ technology for the detection and estimation of heteroplasmy for six mitochondrial point mutations associated with the following diseases: Lebers Hereditary Optical Neuropathy (LHON), G3460A, G11778A & T14484C; Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes (MELAS), A3243G; Myoclonus Epilepsy with Ragged Red Fibres (MERRF), A8344G and Neurogenic muscle weakness, Ataxia and Retinitis Pigmentosa (NARP)/Leighs: T8993G/C. Results obtained from the Nanogen and Pyrosequencing

assays for 50 patients with presumptive mitochondrial disease were compared to those obtained by the current ‚gold-standard' diagnostic technique, PCR and restriction enzyme digestion. Overall, the NanoChip® Molecular Biology Workstation provided accurate genotyping for the six mitochondrial assays but had limitations in determining the degree of heteroplasmy for some mutations. The Pyrosequencing assays provided both accurate genotyping and good determination of mutational load for all mutations. Pyrosequencing also compared favourably when reagent costs and time of analysis were considered. Whilst both systems can be used for detection and quantification of mitochondrial mutations, Pyrosequencing offered a number of advantages in terms of accuracy, speed and cost.

P0645. Spinocerebellar ataxia type (SCA2) associated with white matter affectation

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Spinocerebellar ataxia type 2 (SCA2; 12q23-24) is characterized by slowly progressive ataxia and dysarthria associated with slow saccadi eye movements, as well as relatively high incidence of dystonia or chorea and dementia. SCA2 is caused by a $(CAG)_n$ repeat expansion sequence coding a polyglutamine tract. The pathological range (n) of CAG repeats varies from 35-to >77; the wild type (n) is 15 to 29. The expansions usually increases in size when transmitted to successive generations.
We present an index SCA2 familial case showing white matter affectation and without alfa-sinuclein deposits. Detection of CAG expansion mutation was performed by PCR in a *post-mortem* sample and results in a genotype 22/**36**. We have also identified SCA2 expansions in several familial related individuals. In conclusion, CAG expansion mutations can correlate with white matter affectation in spinocerebellar ataxia type 2.

P0646. The very recently identified type 2A juvenile haemochromatosis gene (HFE2A), a candidate modifier of the C282Y homozygous phenotype

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BACKGROUND: The most common form of haemochromatosis is an adult-onset condition which has mainly been associated with the HFE1 C282Y/C282Y genotype. The phenotypic expression of this genotype is very heterogeneous and depends on a complex interplay of genetic and non genetic factors. Aim of the present study was to determine if mutations in the very recently HFE2A gene were associated with more severe iron overload phenotypes in C282Y-homozygous patients. RESULTS: From a cohort of 310 C282Y-homozygous patients, we found 9 carriers (6 males and 3 females) of an additional HFE2A missense mutation at the heterozygous state (L101P, S105L, E302K, N372D, R335Q or the previously described G320V). Iron indices of these 9 patients appeared to be more severe than those observed in C282Y-homozygous patients of identical sex and similar age ranges. Thus, the mean serum ferritin concentration of the 6 males with a HFE2A mutation was significantly higher than that of C282Y-homozygous males without an additional mutation (2350.3 [s = 1429.9] vs 1227.2 [s = 1130.1] µg/L; p = 0.0233, Student t test). CONCLUSION - DISCUSSION: We have recently reported that mutations in gene that encodes hepcidin could explain one part of the C282Y/C282Y-related phenotypic heterogeneity by accentuating the iron burden. Our new data revealed that mutations in HFE2A could be associated with a similar effect. Taken together, these results emphasized that search for modifier genes could enable us to more precisely distinguish those C282Y-homozygous patients with a higher risk to develop a severe iron overload and, consequently, clinical complications.

P0647. Identification and characterization of a candidate gene in Joubert syndrome

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Joubert Syndrome is a rare, clinically and genetically heterogeneous syndrome, characterized by brain stem and cerebral malformations, hypotonia, ataxia, abnormal eye movements, hyperpnea, neuro-ophthalmologic abnormalities, and mental retardation. The biochemical and molecular basis of JS remains unknown.
We have used flow sorted derivative chromosomes to map the translocation breakpoint in a patient with JS associated with a de novo, balanced translocation t(2;22)(q13;q11.1). We initially generated a microarray containing BAC sets that span chromosomes 2 and 22 at 1 MB intervals. We narrowed the translocation breakpoint to a region between two BACs that are separated by 4MB. Sequence tagged site (STS)-PCR was performed at higher density levels and the breakpoint localized to within a 324 bp region.
The breakpoint on chromosome 2q13 interrupted a number of predicted genes.. Our analysis of a predictive gene cluster has confirmed the presence of a ubiquitously expressed gene, whose precise structure differs somewhat from
any of the members of the cluster. Using 5'RACE and 3'RACE experiments and inter-exon RT-PCR from 28 human tissues, we have found that this gene, named JSC (for Joubert Syndrome Candidate) pro term, contains 10 exons, produces a transcript of ~6 kb and spans a genomic region of over 500kb. The gene appears to be expressed in all tissues examined. There is a large CpG island at the 5'end. The gene has homologues in mouse, rat, fugu and drosophila. We have sequenced all 10 exons of the JSC gene in the patient and have failed to detect any clear mutations.

P0648. Molecular study of Spinocerebellar Ataxias in Iranian Patients

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Objective: To assess the frequency of common types of Autosomal Dominant Cerebellar Ataxia (ADCA), SCA1 (spinocerebellar ataxia type 1), SCA2, SCA 3/MJD spinocerebellar ataxia type 3/Machdo-Joseph disease), SCA 6 and SCA 7, CAG trinucleotide repeat expansion [(CAG)n] among patients clinically diagnosed with hereditary SCA. Identification of the type of ataxia is important to determine the disease prevalence, diagnosis and right treatments for patients.
Materials and Methods: 18 patients from 12 families who clinically diagnosed for SCA were used as subject for this study. Polymerase chain reaction were used to detect (CAG)n repeats in following SCA types: SCA1, SCA 2, SCA 3/MJD, SCA 6 and SCA7. Polyacrylamide gel electrophoresis, product and stained with silver staining. . We are conducting detail mutational analysis by sequencing of PCR products.
Results: Spinocerebellar ataxia type 2 (SCA 1) was identified in 2 families with 6 studied patients. All affected family members were heterozygous for a CAG repeat expansion in the SCA 2 gene, one family with 5 patients showed anticipation. Proband in this family was a 22 years old male who showed ataxia, gait, disturbance and incoordination. Another patient had any family history for SCA. This sporadic phenomenon was found also in other family with SCA type 7.
Conclusion: Because of this is the first report in molecular level about SCA, it's difficult to get conclusion for SCA prevalence, evidence shows more patients who clinically diagnosed with SCA in Iran that Genetic diagnosis could help clinician to make decision for their treatments of patients.

P0649. A change in the phase of segregation in a male CMT1A carrier due to a recombination event within the duplicated region of chromosome 17p11.2

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Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous peripheral neuropathy characterized by gait problems and impaired hand function due to distal paresis and sensory dysfunction. CMT1, the demyelinating form, has an estimated prevalence of 1/2500. We report on a three-generation family in which the father and his mother present with the typical clinical picture of CMT1A. The parents came to see us with their three daughters, 13, 16 and 17 years old, asking for genetic advice. At the time of first contact none of the girls had developed symptoms of CMT1A. In general, CMT1A leads to significant motor disabilities in adult life. Therefore, the parents wished predictive molecular testing in order to help their children to decide for an appropriate profession. Molecular genetic investigations by microsatellite analysis showed that the father and the grandmother share the same characteristic 1.5 Mb duplication on chromosome 17p11.2 containing the peripheral myelin protein 22 gene (PMP22). Analysis of the three daughters demonstrated that two children inherited the nonduplicated grandpaternal haplotype. The youngest daughter presents with a doubled gene dosage of the markers 4A, D17S2228, 9A, 9B and 10A and three unique alleles for the marker D17S2230. Interestingly, the duplication of 1.5 Mb was composed of one grandpaternal and one grandmaternal haplotype. We conclude that a homologous recombination event in the meiosis of the father resulted in a change in the segregation phase of the duplication.

P0650. Hereditary hemochromatosis: genotype/phenotype correlations for the main HFE mutations

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Characterized by iron overload, hereditary hemochromatosis (HH) is the most common autosomal recessive disorder in Caucasian populations. It is a complex disorder whose phenotypic expression results from interactions between genetic and environmental factors. A candidate gene for HH (HFE) was cloned in 1996, including a main mutation (C282Y), two susceptibility factors (H63D, S65C) and a tenth of private mutations. This study aimed to compare the phenotypic expression of the main HFE genotypes.
We retrospectively analyzed a cohort of HH patients treated in western Brittany, France. A clinical questionnaire, completed at the time of onset, provides information on biochemical parameters, clinical data, genotype and treatment.
This study included 585 patients: 433 C282Y/C282Y, 114 C282Y/H63D and 38 H63D/H63D. Age at onset and iron parameters values (serum iron, serum ferritin, transferrin saturation) were significantly lower in the two last groups (respectively, $p=0.02$ and $p<0.001$ for each parameter). The C282Y/H63D and H63D/H63D genotypes were associated with lower frequency of arthritis ($p=0.0012$) and skin pigmentation ($p<0.0001$) in comparison with the C282Y/C282Y genotype. Moreover, higher proportions of metabolic disorders ($p<0.0001$) and high-blood pressure ($p=0.02$) were observed in patients carrying those genotypes. Comparison of the C282Y/H63D and H63D/H63D genotypes showed no significant difference, excepted a significantly higher proportion of skin pigmentation among the H63D-homozygous patients ($p=0.028$). This study highlights the genotype/phenotype correlations for the main HFE genotypes. The results confirm that the two less common HFE genotypes are associated with milder forms of HH, and show that the patients carrying those genotypes present more frequently metabolic disorders.

P0651. Large genomic APC deletions are a frequent cause of familial adenomatous polyposis

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Familial adenomatous polyposis (FAP) is an autosomal-dominant precancerous condition of the colorectum that is caused by germline mutations in the tumour suppressor gene APC. Most mutations reported in the literature are point mutations. Large genomic deletions might account for a substantial number of patients. Indeed, earlier we had detected large deletions in 4 FAP families based on apparent nonpaternity of intragenic and closely flanking polymorphic markers. Here we present results of a systematic search for large deletions by use of MLPA (multiplex ligation-dependent probe amplification). The MLPA test kit (MRC Holland) contains 20 paired probes spanning the entire APC region. We could characterise the 4 deletions detected by linkage analysis (2 deletions of the complete APC, one deletion of exons 8-15 and one deletion of exons 14-15). Subsequently, we examined a subset of 49 unrelated patients in whom no mutation had been detected by PTT of exon 15 and DHPLC of exons 1-15B, and identified large deletions in another 14 patients. Eight of the patients were identified with different partial deletions encompassing one up to several exons, while the remaining 6 patients had deletions of all 20 fragments. The relatively high proportion of large deletions in this first subset of patients (28%) may be due to a selection of patients for an autosomal-dominant mode of inheritance and typical FAP, part of them showing no heterozygozity for intragenic polymorphisms.
The study was supported by the German Cancer Aid (grant 70-2783-Fr 1).

P0652. Molecular genetic characterization of intrahepatic cholestasis of pregnancy

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Intrahepatic Cholestasis of Pregnancy (ICP) is a disorder which complicates otherwise normal pregnancies, increasing risk of fetal loss or preterm delivery. Prevalence varies between populations (0,01% USA, 27,6% Chile).The etiology of ICP is multifactorial including genetic, hormonal and environmental factors. This study investigated molecular genetic factors possibly involved in development of ICP in Greece: Multi-Drug Resistance gene 3 (MDR3); Uridino-glucuronosyl-transferase (UGT1A1) gene; and Estrogen Receptor genes (ERalpha, ERbeta). MDR3 encodes a P-glycoprotein mediating translocation of phosphatidylcholine across the canalicular membrane of hepatocytes; mutations in MDR3 have been observed in ICP. The UGT1A1 gene encodes uridino-glucuronosyl-transferase which mediates steroid metabolism. Estrogen receptors are ligand-activated transcription factors that mediate action of estrogen in target tissues. In 10 Greek women with clinical and biochemical findings of ICP and 45 Greek pregnant women without ICP (controls) we investigated the presence of 4 MDR3 gene mutations previously observed in patients with Progresssive Familial Intrahepatic Cholestasis and/or ICP (ex6 7bpdel, ex14 cd546C>A and 1712delT, ex23 cd957C>T). Additionally we investigated: distribution of microsatellite allele-sizes linked to the UGT1A1 gene (TA repeats in promoter), the ERalpha gene (TA repeats in promoter) and the ERbeta gene (CA repeats in intron 5). No MDR3 gene mutations were found in any case. For the UGT1A1 gene, 20% of ICP cases vs 16% of non-ICP cases had the (TA)7/(TA)7 genotype. Microsatellite allele-size distribution of the ERalpha and beta genes did not show obvious differences between the two groups. Overall conclusive results are precluded by the small number of cases studied so far.

P0653. Molecular analysis of a translocation t(17;20)(q25;q13) in a patient suffering from SRS

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A severe Silver-Russell syndrome (SRS), a heterogenous disorder characterized by growth retardation, lateral asymmetry and other dysmorphologies, ascertained in a girl with a translocation t(17;20)(q25;q13), was reported by Ramirez-Dueñas et al. (1992). Here we report the molecular analysis of both breakpoint regions based on specific PAC and BAC clone contigs. Several clones could be identified giving signals on both chromosomes 17 and der17 in FISH analysis using proband metaphase chromosomes so establishing a refined clone contig for the 17q25 region. According to the mutation analysis of the candidate region for hereditary neuralgic amyotrophy (HNA; Meuleman et al., 2001) we could localize the gene SEC14L1 nearby the region of interest by PCR approach. STS content mapping and sequencing of the clone insert ends resulted in locating the breakpoint within 81,5 kb in the 5´- region of SEC14L1. Corresponding FISH analysis resulted in the identification of a PAC clone RP1-232n11 spanning the breakpoint on chromosome 20 including parts of the Receptor Protein Tyrosine Phosphatase Rho gene (PTPRT) considered to be another SRS candidate gene. In order to establish a precise physical map of the region of interest we located one XhoI fragment of RP1-232n11 distal to the 20q13 breakpoint. Further experiments using other clone subfragments should lead to a high resolution map of the 20q breakpoint region thus giving novel information on the genetic background of SRS.

P0654. Molecular characterisation of 20 patients with Cohen syndrome: Spectrum of mutations in COH1

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Cohen syndrome is a rare autosomal recessive disorder, highly variable and mainly characterized by mental retardation, microcephaly, truncal obesity, short stature, craniofacial features, myopia, retinal dystrophy, and neutropenia. Mutations in a novel gene, COH1, on chromosome 8q22 were recently reported in Cohen syndrome patients from Finland and other parts of Northern Europe. Here we describe molecular and clinical findings in 20 patients with Cohen syndrome from twelve families, originating from Brazil, Germany, Lebanon, Oman, Poland, and Turkey, with mutations in COH1. We identified a total of 17 different novel mutations, including nine nonsense mutations, six frameshift mutations, and two potentially pathogenic missense mutations. In a Lebanese family with an overlapping phenotype of Cohen and Mirhosseini-Holmes-Walton syndromes we found a mutation in the splice acceptor site of intron 51 resulting in activation of a cryptic splice acceptor site in exon 52 and a lack of 16 bp mRNA sequence. Patients from four consanguineous families and one family without known consanguinity carried homozygous mutations, all others were compound heterozygous. All mutations were found only once, distributed throughout the coding region of COH1 as far as known today. Our findings demonstrate that Cohen syndrome may be caused by mutations in COH1 also in patients from outside Northern Europe with a clinical picture diverging from the homogeneous phenotype of Finnish patients. A consistent genotype/phenotype-correlation, however, has not been found so far. The COH1 product appears to have a certain tolerance to residue substitutions. These data indicate that COH1 is subject to wide allelic heterogeneity.

P0655. Molecular diagnosis of Cystic Fibrosis in Belarus: Update

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The incidence of cystic fibrosis (CF) in Belarus is about 1:8000 newborns. Since 1991 a total of 131 unrelated Belarus families with at least one affected subject have been investigated. Diagnostic criteria involved positive sweat tests (Cl > 60 mmol/l) and typical clinical findings of pulmonary and gastrointestinal disease.
DNA diagnostics by means of detection of the dF508 mutation began in 1991. Since this period the number of mutations routinely screened in CF patients gradually increased to 12, analysed by ARMS, restriction and gel retardation tests. Eleven mutations have been found in 262 CF chromosomes with relative frequencies as follows: dF508 - 60.8 %; CFTRdele2,3(21kb) - 7.0%; 2184delA - 3.7%; N1303K - 3.5 %; 3849+1kbC-T - 1.9%; W1282X - 1.6 % G542X - 1.2%; R334W - 0.8%, R553X, R347P and S549N - 0.4%, giving overall detection rate of 82.7 %. Our data show that CFTRdele2,3(21kb) mutation, particularly common in Eastern Europe, has a higher frequency in Belarus than in any other population.
First trimester prenatal diagnosis has been performed in 38 CF families by means of direct mutation analysis and combined analysis using the intragenic polymorphism IVS6a-GATT in the CFTR gene. Eight affected, 18 heterozygous and 12 normal fetuses were detected.
We are continuing analysis, in order to identify other mutations in so far uncharacterised CF alleles. The knowledge of the spectrum of mutations causing CF in our country would provide useful information to design the best approach in pre and postnatal diagnosis of CF and for population screening.

P0656. The axonemal intermediate dynein gene DNAI1 and asthenozoospermia

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The axonemal intermediate dynein gene DNAI1 is mutated in Primary Ciliary Dyskinesia (PCD), an hereditary disorder characterized by recurrent pulmonary and upper respiratory tract infections. This disease is due to immotility of cilia covering the respiratory epithelium. Since the ultrastructure of the cilia is almost identical to the ultrastructure of the flagellum, these patients have often immotile spermatozoa (asthenozoospermia). It may be possible that different mutations in the DNAI1 gene lead to different phenotypes. To investigate this hypothesis we performed a mutation search in the DNAI1 gene in 20 infertile asthenozoospermic men without respiratory problems.In three patients we detected three different base changes never described before. Two of these variations change the aminoacid. In exon 1 a G>T introduces a new GT donor splice in the open reading frame and changes the Ala in position 8 into a Ser (A8S). In exon 19 a C>T changes the arginine 650 in a cystein (R650C). DHPLC analyses showed that these two alleles are not present in 50 fertile men from the same ethnic origin and in 30 italian PCD patients. The above variations are both in heterozygosity and they are not frequent polymorphisms. If this trend will continue we will find that changes in DNAI1 gene are present in 10% of asthenozoospermic patients. We will perform electron microscopy and functional studies to assess the pathogeneity of these changes.It is important to discriminate between environmental and genetic asthenozoospermia, in order to allow responsible reproductive choices to carriers of a genetic condition.

P0657. Mutation search in the intermediate axonemal dynein gene DNAI1 in 30 Italian patients with Kartagener Syndrome

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Primary Ciliary Dyskinesia (PCD) is a rare (1:20000) heterogeneous hereditary disorder characterised by immotility of cilia, leading to recurrent pulmonary and upper respiratory tract infections. The chronic 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 recurrent respiratory infections, ciliary dyskinesia and situs inversus are present, the pathology is named Kartagener Syndrome (KS). The ultrastructure of the cilia is almost identical to the flagella's one and patients' spermatozoa often are vital with immotile flagella (asthenozoospermia).
We collected 30 unrelated KS/PCD patients 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 to date analysed has a dynein arm deficiency.We searched the patients' DNA for mutations in the genes in which mutations leading to PCD/KS have already been described: DNAI1, an intermediate dynein chain, DNAH5 and DNAH11 two heavy chain axonemal dyneins. We finished the analysis of the entire DNAI1 gene (20 exons and flanking intronic regions) and no pathogenetic alterations were found. Our findings show that in Italy PCD/KS is almost never due to mutations in the DNAI1 gene (less than 3,3%). Preliminary data on DNAH5 and DNAH11 show that the previously reported mutations are not frequent in Italy. It is possible that the Italian patients share the same mutated gene/s which is to be individuate.

P0658. Functional analysis of mutant alleles identified in Spanish mucopolysaccharidosis IIIA (Sanfilippo A) patients

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Mucopolysaccharidosis IIIA (MPS IIIA) is an autosomal recessive lysosomal disorder caused by the deficiency of sulfamidase (SGSH; EC 3.10.1.1), which is needed for the heparan sulfate catabolism. Approximately, 70 mutations have been identified in different countries as responsible for MPS IIIA. Our group recently reported the molecular defects of 26 unrelated Spanish patients. In order to demonstrate the causality for MPS IIIA of some of these mutations, we expressed 8 out of the 14 mutant alleles present in that group of patients and the two variants of the polymorphism p.R456H. In particular, we expressed the mutant enzymes carrying changes: p.S66W, p.R74H, p.Q85R, p.R206P, p.L386R, p.R433Q, p.R433W and c.1079delC, using a baculovirus expression system. The expression and further characterization revealed that the activity of the majority of these mutant alleles were severely compromised, presenting only 1-2% of the wild type enzyme activity. Only mutations p.S66W and p.R206P showed some levels of activity, 10% and 18% of wild type enzyme, respectively. In all cases, we detected the presence of the expected two forms of the sulfamidase, the precursor and the mature proteins, indicating a normal processing of the mutant enzymes.

P0659. Genome-wide homozygosity search in two brothers with Kartagener Syndrome

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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, which often result in bronchiectasis, and progressively damage the lungs (with need of transplant). Dextrocardia, with or without situs inversus, is present in 50% of the patients. If respiratory infections, ciliary dyskinesia and situs inversus are present, the pathology is named Kartagener Syndrome (KS). Often patients' spermatozoa are vital with immotile flagella. Cilia and flagella have in common the axoneme, a structure given by about 250 different proteins. A deficit in any one of them may have pathological consequences. Here we present two KS brother that don't show mutations in the genes already associated to KS (the axonemal dyneins DNAI1, DNAH5 and DNAH11) and lack outer dynein arms. Their parents have a common ancestor. We assumed that KS is due to homozygosity of a mutated alleles identical by descent (IBD). We performed a genome-wide microsatellite analysis using 180 markers and we found the brothers share the same chromosome X and are homozygote for chromosomes 20, 17p, 19ptel, and few other markers. We focused our candidate gene search in these regions. Two heavy chains dynein genes map in the short arm of HC17. One, DNAH2, has only been annotate (and we are cloning it), while the other, DNAH9, consists of 69 exons. To date we analysed more than 50 DNAH9 exons, we found some variants in homozygosity but none of these changes seems to be pathogenetic.

P0660. Search for structural rearrangements of the *HBII-85* snoRNA gene cluster in patients with atypical PWS

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Prader-Willi Syndrome (PWS) is a neurogenetic disorder caused by the loss of function of one or more imprinted genes in 15q11-q13, which are paternally expressed only. Some of the genes encode proteins, whereas others encode small nucleolar RNA (snoRNAs). Paternal deletions in mice have pointed to the presence of a gene or genes between *Snurf-Snrpn* and the *Ipw* exons, which may contribute to the PWS phenotype. Based on its location, the *HBII-85* snoRNA gene cluster, which consists of 27 gene copies, is a potential candidate for this.
In three patients with atypical PWS and a balanced translocation, the translocation breakpoints are located in exon 20 of *SNURF-SNRPN.* In one case studied, loss of *HBII-85* expression was observed. Since repeated DNA sequences can be targets for recombination events, it is possible that patients with some features of PWS have a rearrangement or deletion of the *HBII-85* cluster. To address this question, we first screened 11 patients with atypical PWS by Southern blot analysis and found no evidence for a deletion or a rearrangement. To screen a larger cohort of patients, we have established a multiplex amplifiable probe hybridization (MAPH) assay, which allowed us to detect copy number changes at nine different positions inside the 55 kb *HBII-85* snoRNA gene locus in one hybridization. A locus from chromosome 8 served as a control. So far, in 94 patients with mental retardation and obesity, no deletion could be detected.

P0661. Screening for genomic rearrangements involved in non-syndromic X-linked mental retardation

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Genomic rearrangements play an important and hitherto underestimated role in the etiology of human genetic disease. Very often, such rearrangements are mediated by non-allelic homologous recombination (NAHR) between low-copy repeats (LCRs). In one of our recent studies aiming at the detection of all causative mutations in families with non-syndromic X-linked mental retardation (NS-XLMR), we have found in only 1/3 of the families tested functionally relevant mutations. It is conceivable that some of the missing mutations may not be detectable by point mutation screening, such as submicroscopic rearrangements due to NAHR between LCRs. This has prompted us to search for possible genomic rearrangements in patients with XLMR. *In silico* studies conducted in our group have revealed 435 nearly identical LCRs, which are present on the X chromosome in at least two copies. Due to possible incomplete and incorrect assembly of genomic sequences some of the computationally identified duplicated segments might be artifacts. Therefore, we have experimentally proven (by PCR on male DNA and subsequent sequencing) that 32 out of 80 putative X chromosome specific duplications show apparent heterozygosity, indicating their duplicated nature. Additionally, we could independently confirm these results by Southern Blot hybridization. Analyzing the genomic regions flanked by these 32 duplications we have identified 39 candidate genes, which could be affected by possible rearrangements. These genes are being investigated in patients from XLMR families with overlapping linkage intervals by gene expression studies, by multiplex amplifiable probe hybridization (MAPH) and by multiplex ligation-dependent probe amplification (MLPA).

P0662. Isolated COX-deficiency - broad clinical spectrum caused by mutations of nuclear and mitochondrial genes

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Isolated deficiency of cytochrome c oxidase (COX) is a common cause of respiratory chain disorders (1:20 000). It is frequently

associated with severe symptomes like Leigh disease, hypertrophic cardiomyopathy or fatal cardioencephalomyopathy of infancy, usually caused by defects of nuclear-encoded COX assembly genes (SURF1, SCO2, SCO1, COX10, COX15). A slowly progressive late-childhood onset myopathy with variable encephalopathy can be caused by mutations of the mtDNA encoded COX subunits (COI-III). In order to establish genotype-phenotype correlations we present the molecular and clinical findings in our collective of 72 patients with isolated COX deficiency. Most frequently COX deficiency is caused by mutations of SURF1. We summarise the clinical and genetic findings of our 15 patients carrying mutations in SURF1. SCO2 encodes a protein transporting Cu++ to COX. A common (E140K) mutation was found either compound heterozygous or homozygous in all patients described so far. Seven of our 8 cases developed cardioencephalomyopathy with death in the first weeks (heterozygous patients) or first year (homozygous patients) of life. In one homozygous patient substitution with copper-histidinate was associated with a recovery of cardiomyopathy, however she died at age 39 months of pneumonia. Two cases with mutations in COX10 presented with Leigh-like disease and anaemia. A stop-mutation (W58X) in mtDNA-encoded COIII was found in a patient presenting at age 12 years with a non-progressive mild myopathy. Mutations in tRNASer were detected in 5 cases causing progressive myoclonus epilepsy and myopathy. Summarising our findings we provide a diagnostic strategy in cases with isolated deficiency of COX.

P0663. Increased polymorphism of the mitochondrial DNA polymerase gamma gene (POLG) among astenozoospermic patients.

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Infertility in humans affects between 10 and 15% of all couples at reproductive age. Different genetic defects have now been identified in an important proportion of infertility cases in man. Exogenous factors such a infection or trauma explain an additional number of cases of male infertility. However, still today, in the majority of cases of male infertility or subfertility a genetic or environmental cause can not be identified. In the present work we have focussed on the POLG polymorphism as potential genetic risk factor. Two hundred and forty seven patients and sixty four blood donors were included in this study. The POLG polymorphism was determined essentially as previously described (Rovio et al, Nat. Genet., 2001, 29 (3): 261-262). The wild type genotype was found in more of an 80% in all groups studied. The heterozygous mutant frequency was increased from 6% in controls to 10-12% in the different infertile groups although the differences were not statistically significantly. However a significantly increased frequency was found in the homozygous POLG mutant in the asthenozoospermics group (6%) as compared to the non-asthenozoospermics patients (0.5%). This results could be related to the energy related function or mitochondria. Supported by grants from Ministerio de Ciencia y Tecnologia (BMC 2003-03937), fondos FEDER, Ministerio de Sanidad y Consumo (V-2003-REDCO7A-O) and by Generalitat de Catalunya (2001 SGR00382) to R.O.

P0664. Spinocerebellar Ataxia Type 7. Correlations Between Phenotype And Genotype In 8 Spanish Families.

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Spinocerebellar ataxia type 7 is an autosomal dominant cerebellar disorder with retinal degeneration.
Symptoms are dysarthria, dysphagia, ataxia and progressive visual impairment. The expansion of a coding CAG repeat in the SCA 7 gene causes this disease and displays as in other neurodegenerative conditions with marked anticipation of both onset age an rate of progression.
In an extensive genetic study of more than five hundred Spanish families affected with ataxia we conclude that eight of them present an increased number of repeats in the SCA 7 gene. Of all dominant ataxias for which a genetic cause could be established, we have found SCA 7 to be the second most prevalent form in our population. The first neuropathological sign was gait ataxia with visual failure, and the pathological repeat range was between 35-55 repeats.
The tendency for earlier onset and increased phenotypic severity in younger generations (anticipation) is
clearly demostrated. There are juvenile cases resulting in death (5-7 years) in three families with paternal allele transmision. In these cases the paternal allele size was greater than 41 repeats. In siblings of the same generation the youngest patients present larger alleles and this correlates with an earlier onset and more severe phenotype. Five of these eight families share the same geographical location (Guadalajara), three of wich are those aforementioned families presenting juvenile cases resulting in death. Therefore, a common genetic origin is strongly suspected. Further linkage analysis could provide evidence of founder effect.

P0665. Simultaneous investigation of five polymorphisms associated with cardiovascular disorders in a multiplex PCR reaction combined with fluorescent minisequencing

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Here we descriebe a novel approach for simultaneous screening of five polymorphisms associated with cardiovascular diseases.
The Leiden mutation of coagulation factor V is a significant risk factor for thromboembolic diseases; G202210A mutation of coagulation factor II (prothrombin) is of low frequency, and it is a risk factor mainly in association with Leiden mutation. The C677T polymorphism of MTHFR gene was found to correlate with the plasma homocysteine level, elevated plasma homocysteine being a risk factor for coronary-artery disorders. T196C polymorphism of the gene encoding glycoprotein IIIa (PLA) may cause disorders of platelet binding, and was found to correlate with the occurrence of acut coronary syndrome (ACS). The C-453A polymorphism in the promoter region of the fibrinogen gene can be associated with elevated fibrinogen level, being a risk factor for ischemic cardiac disease.
We elaborated a multiplex PCR reaction combined with fluorescent minisequencing (SNaPshot), for simultaneous detection of the mutations mentioned above, using the ABI Prism 310 Genetic Analyzer. We think this is a cost-effective and powerful tool for conducting correlation studies in a target population.

P0666. Maternal Transmission of a New Mutation in a Family with Mild Sotos Syndrome

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Sotos syndrome is characterized by mental retardation, pre- and postnatal overgrowth, and typical facial features. For nearly forty years Sotos syndrome had to be diagnosed on the basis of clinical features of which the most important was the facial gestalt, a rather vague criterion which may explain a diagnostic overlap with Weaver syndrome.
In 2002 came a sudden shift in interest from the clinical specialist to the molecular geneticist when Kurotaki et al showed a microdeletion of the NSD1 Gene on one chromosome 5q35 in 45/112 individuals with a clinical diagnosis of Sotos syndrome and an intragenic mutation in another 16/112. These findings could only partly be reproduced in Caucasians in whom intragenic mutations of the NSD1 gene were more common and larger microdeletions seemed to be rare.
So far, only four familial cases with molecular studies have been published , a finding that supports clinical observations of a low recurrence risk in siblings and children.
We investigated a family with two affected children and their likewise affected mother with typical clinical features, especially overgrowth but a remarkable intellectual catch-up, which enables them to attend normal school and work as a skilled craftsperson, respectively.
Sequence analysis revealed a missense mutation 6523T>A, which causes a change from cystine to serine in the protein.
Interestingly its position in exon 23 is close to the deletion

6532delTGCCCCAGC reported in a familial case by Türkmen at al (2003).

P0667. X-linked Juvenile Retinoschisis In 14 Spanish Families: Identification Of 4 Novel Mutations

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Introduction: X-linked juvenile retinoschisis (XLRS) is a disorder characterized by bilateral macular involvement with onset on the first decade of life. Affected males show areas with splitting of the nerve fiber layer of the retina ("schisis") and presence of vitreous veils, giving the aspect of a "spoke wheel" pattern.
The RS1 gene maps to Xp22.2-22.1, has 6 exons and encodes a 224 amino acid protein with a highly conserved discoidin domain, implicated in cell-cell adhesion, being very important during retinal development.
Objective: Identification of mutations in RS1 gene in 14 Spanish families clinically diagnosed of XLRS.
Patients and Methods: We studied 14 unrelated Spanish families. The 6 exons of the RS1 gene were amplified by PCR and sequenced in an ABI PRISM 310. Restriction assays were performed to confirm novel mutations (when possible). Haplotype analyses with STRs were also performed.
Results: We could identify 7 previously described mutations in 9 families (E72K in 3 families) and 4 novel mutations in 5 families (Q154R in 2 families). Two of them were "de novo".
Conclusions:
· High frequency of "de novo" mutations: the site of mutations are mainly in CG dinucleotides.
· Haplotype analyses result very useful for showing that E72K mutation arose independently in different
families (hot spot).
· However, Q154R mutation was identified in 2 families and they share haplotypes, so we can not rule out
identity by descent.

P0668. Mutational screening of the *ARX*, *MECP2* and *FMR2* genes in a series of 200 male patients with mental retardation.

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X-Linked Mental Retardation (XLMR) is a genetically heterogeneous condition which can present in either syndromic (MRXS) a nonspecific (MRX) form. The *ARX* and the *MECP2* genes were found mutated in both MRXS and MRX cases, while expansions in the *FMR2* gene are usually associated with MRX. We screened 200 male patients with mild to severe MR for mutations in the *ARX*, *MECP2* and *FMR2* genes. All patients were negative for the amplification in the *FMR1* gene, thus excluding fragile X syndrome, and none of them had dysmorphic features. Only few (7%) had a history of seizures or infantile spasms. *ARX* mutations were screened by SSCP, using 8 primer pairs designed by us (available upon request). We found 4 cases with a silent point mutation in exon 4 (G1347T), one with a G>T transversion in intron 3 (position +81) and one *de novo* case with the 24-bp duplication previously described by Stromme et al. [Nature Genet (2002) 30:441-445]. This latter patient was a 4-year old child with dystonic movements of the hands, typical of Partington syndrome. The *MECP2* gene was analyzed by direct sequencing of the entire coding sequence. We only found 5 silent point mutations in exon 3 and one G>C transversion in intron 3 (+30). Finally, no expansion was detected in the CGG repeat of the *FMR2* gene. Our results suggest a minor role for the *ARX* gene in causing nonspecific XLMR and indicate that other yet unidentified genes wait to be characterized.

P0669. Rapid detection of CFTR gene deletions and rearrangements by semi-quantitative fluorescent multiplex PCR and implications for genetic counselling

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Cystic fibrosis (CF), one of the most frequent hereditary diseases in the Caucasian population, is mainly due to point mutations scattered over the whole cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. *CFTR* gene deletions are rare but their frequency may be underestimated because missed when using conventional PCR techniques. We have developed an assay based on semi-quantitative fluorescent PCR targeted on the 27 *CFTR* exons in a three multiplex-format to screen for unknown rearrangements in 62 CF patients or parents of CF patients bearing one or two unidentified CF alleles after an extensive *CFTR* gene study. We characterized eight different rearrangements in eleven patients, of which all but one are new: CFTRdele1-24, CFTRdele1, CFTRdele2, CFTRdele3-10,14b-16, CFTRdele14b-17b, CFTRdele17a-17b, CFTRdele17b and CFTRdup4-8. Taking into account the previously characterized CFTRdele2-3, CFTRdele17a-18 and CFTRdele19 anomalies in four other CF patients, gene rearrangements thus account for 19% of unidentified CF alleles and of almost 1% of total CF mutations in our population. These results suggest a hotspot for deletions at the IVS17b(TA) microsatellite. Given the feasibility and reliability of the method here described and its sensitivity to detect large rearrangements as well as single nucleotide deletions/insertions, which altogether account for 23% of the CF mutations described, its place in the strategy for *CFTR* studies is worth to be considered in the second line after the screening for frequent mutations. The availability of such a tool has notable implications for patients' care and genetic counseling for the patients and their family.

P0670. Mutation analysis of ATP7B gene in Wilson disease patients of Turkish origin

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Wilson disease (WD), an autosomal recessive disorder of copper transport results from mutations in the ATP7B gene. To date more than 200 mutations of the ATP7B have been identified. In the last years we have analyzed the molecular basis of WD in the mediterranean and suggested strategies of molecular testing in specific populations. In this study we summarize the results of mutation analysis of the ATP7B gene in 71 WD families originating from Turkey. Using the SSCP and sequencing methods we have characterized the 81% of the analyzed chromosomes and identified 44 WD causing-mutations (32 missense, 6 frameshift, 3 splice, 3 nonsense) suggesting a high allelic heterogeneity for WD in this population. The most frequent mutation Arg778Gly, is only present in the 7.7% of the analyzed chromosomes. Most of identified mutations i.e. 78.2% reside in 10 exons, 2[II], 8, 10, 12, 13, 14, 15, 17, 18, 19 of the ATP7B gene. In 40/71 analyzed families, mutation was identified in the homozygous state suggesting a high degree of consaguinity. The presence of a few number of homozygotes for a specific mutation doesn't permit a genotype-phenotype correlation study. Our study suggests that molecular analysis of ATP7B in Turkish WD patients should include as firs step the analysis of the ten exons reported above, and subsequently the remaining gene. These data can be used for a strightforward geneting study not only in Turkey but also in other countries composed of genetically mixed populations like the USA and other West European countries.

P0671. Genetic analysis of the oculo-auriculo-vertebral spectrum (OAVS)

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The oculo-auriculo-vertebral spectrum is a developmental disorder

characterised by hemifacial microsomia, epibulbar tumors, ear malformations and vertebral abnormalities. Most cases are sporadic, but rare familial cases suggest that OAVS has a genetic basis.
We studied a patient with OAVS and multiple exostoses.
Chromosome analysis of this patient revealed a translocation 46,X X,t(4;8)(p15.3;q24.1). The chromosome 8 breakpoint disrupts the multiple exostoses gene *EXT1*. Since there is no evidence for a gene causing OAVS on 8q24.1, we cloned the chromosome 4 breakpoint. This breakpoint disrupts the *RAB28* gene. We analysed *RAB28* in 75 patients, but did not find any mutation.
The *BAPX1* gene maps 76.4kb proximal to the chromosome 4 breakpoint. Although the gene is not disrupted itself, the translocation disrupts the evolutionarily conserved linkage group of the homeobox genes *HMX1*, *MSX1* and *BAPX1*. Previous studies in mice and Xenopus have shown a role of *BAPX1* in craniofacial development, which makes *BAPX1* a good positional candidate gene for OAVS.
We screened 92 patients for *BAPX1* mutations. In 10 patients we found 6 different rare nucleotide changes, 3 with and 3 without an effect on the amino acid sequence. In 2 families the amino acid changes were also found in non-affected relatives.
Whereas *BAPX1* is biallelically expressed in normal fibroblasts, we found monoallelic expression in fibroblasts from one patient. It is tempting to speculate that epigenetic silencing of one allele may cause OAVS.

P0672. Molecular analysis in 163 unrelated patients with albinism: Mutations in the genes for OCA1, OCA2, OCA3, and OCA4

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Human oculocutaneous albinism (OCA) is a clinically and genetically heterogeneous disorder that affects approximately 1 in 20,000 individuals. Defects in the melanin biosynthesis or transport result in little or missing pigment. To date, mutations in four genes have been identified in patients with recessively inherited oculocutaneous albinism. Here we report the first analysis of a large West European DNA sample collection for the tyrosinase gene (OCA1), the P gene (OCA2), the tyrosinase related protein-1 gene (OCA3), and the MATP gene (OCA4) of 163 unrelated patients.
For the OCA1 gene, we found 78 different DNA variations in 82 individuals. Among these are 68 nucleotide substitutions resulting in amino acid changes, stop mutations and polymorphisms as well as four nucleotide insertions and six deletions. Furthermore, we found an accumulation of three to five mutations in 17 patients with OCA1. Results of the OCA2 gene analysis revealed three patients who carry two mutations in this gene. For 18 patients single mutations have been detected. Interestingly, 10 patients carried mutations within the OCA2 gene as well as within the OCA1 gene. One patient showed a combination of an OCA2 plus an OCA3 mutation. In addition to single mutations in the OCA3 gene in five patients, we detected polymorphisms for this locus. Finally, we identified five individuals with two and six cases with a single OCA4 mutation. These include ten so far unknown OCA4 mutations. Overall, we were able to detect mutations in 63% of patients.

P0673. „Burnt-out“ phase hypertrophic cardiomyopathy and systolic dysfunction : association with MYBPC3 gene mutations.

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Objectives: We studied the clinical and genetic features of hypertrophic cardiomyopathy (HCM) caused by mutations in the Cardiac Myosin-Binding Protein C gene (MYBPC3) in italian patients. Background: Mutations in MYBPC3 represent the cause of HCM in about 15% of familial cases. Studies have shown that HCM caused by MYBPC3 mutations may have a delayed onset and is usually benign. Methods: All 35 exons of MYBPC3 were screened by DHPLC followed by automatic sequencing. The diagnosis was based on two-dimensional echocardiographic identification. Results: Overall, we identified 22 MYPC3 mutations in 19 patients (a 15% prevalence). Fifteen mutations (68%) were novel (IVS18+1C>T, A522T, V771M, E165D, ins/del exon 25, V189I, G531R, D786Y, R273H, E334K, R470W, Y340X, A693S , IVS12+1G>A, IVS24-2A>G) and not detectable in 100 normal controls, thus excluding the possibility of polymorphisms; three patients (16%) had two mutations affecting MYPC3. Average age at diagnosis in the 19 patients was 41.3±14.6 years;8 (42%) were female.Of note, evidence of systolic impairment (ejection fraction <0.50) was present in a high proportion of patients (n=5, 23%; p<0.05 versus the overall study group).Conclusions: In a regional HCM population from Central Italy, MYBPC3 mutations were common (15% of unrelated patients); were associated with a broad spectrum of clinical and echocardiographic manifestations, with a high prevalence of systolic impairment; and had different mechanisms (missense, nonsense, insertion/deletion, splice-site). Most of the mutations were novel including those found in the three patients with compound heterozygosis.(Carrier et all., 1997, Circ Res, 80:427).

P0674. Real Time PCR for carrier screening of the commonest mutations of Beta-Thalassemia in a Northern Italy Region

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The haemoglobin disorders interesting the production of beta globin chains are markedly high in several regions of Italy. The most common beta thalassemia mutations found in Italy are Cd39 (C->T) and IVSI-110 (G->A), accounting for 40% and 23% respectively of microcytemic individuals. To know the frequency of carriers of these mutations in our country, we tested DNA samples of 828 schoolchildren aged 13-15 years, resident in the southern of the Verona province (Italy). The gold standard method we chose was a multiplex PCR based on ARMS principle and visualisation of amplicons by UV transillumination of agarose gel. We also optimised a multiplex PCR for rapid screening for these mutations utilizing a procedure based on Real Time PCR and SYBR GREEN detection. Moreover we could compare several modern PCR-Real Time analysers: a) Bio-Rad i-Cycler, b) Stratagene Mx3000, c) MJR MJ Opticon2 . In the children studied we found 27 carriers of mutations (3,26%). 17 cases (2,05%) were found etherozigouse for Cd39 while 10 cases (1,21%) resulted as carriers of IVSI-110 mutation. Our results have evidenced a non elevated frequency of these molecular defects, contrasting with a higher percentage of microcytemic subjects.
We are extending the genetic analysis to other mutations not investigate in the present study that might be associated with microcytemia. To this purpose the employ of the Real Time PCR was found very useful. Furthermore the method we have optimised is not expensive, while is particularly adapted for rapid analysis in a large number of individuals.

P0675. Prevalence of mutations in the CFTR gene among patients with idiopathic chronic pancreatitis

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Recent evidence of mutant CFTR alleles being associated with chronic idiopathic pancreatitis (CIP) indicated that in some patients this may be a monosymptomatic atypical mucoviszidosis. In the present study we apply for the first time sequencing of the whole coding region of the CFTR gene next to screening for frequent CF mutations to allow a precise estimation of the prevalence of CF alleles.66 patients, aged between 5 and 63 years with CIP were included in the study. All patients were screened for 86% of the CF mutations in the Caucasian population. The heterozygous or negative patients were then subjected to sequencing analysis.
Twelve (18.2%) of the patients were detected to carry mutant CFTR allele(s). Compound heterozygosity for F508del and another CF mutation was present in three patients (4.5%) and nine (13.6%) were heterozygous for one (rare) mutation, not detectable with the screening panel.
The incidence of atypical mucoviszidosis in our patient group is 1:

22 and the prevalence of CF carriers is 1:7, i.e. significantly higher than in the normal population. These findings support the evidence for association of mutant CFTR alleles with CIP. Screening for the common CF mutations would be therefore indicated, especially in adolescent patients. The elevated heterozygosity rate for rare CF changes is of importance in terms of family planning (higher risk for a CF affected offspring). Most of the mutations in our cohort will be missed by the screening panels and require more intensive analyses.

P0676. ABCR gene mutations related-Stargardt disease in Italian patients.

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Purpose:Stargardt disease (STGD) is a progressive juvenile-to-young adult-onset macular degeneration, characterized by severe reduction of central visual acuity and normal peripheral vision. STGD is predominantly inherited as an autosomal recessive trait; even if autosomal dominant forms have been described. Homozygous and compound heterozygous mutations in the ABCR gene are responsible for recessive Stargardt macular dystrophy. In this study we determined the mutation spectrum in the ABCR gene in a group of Italian patients with autosomal recessive Stargardt disease .Methods:Thirteen families from central Italy , some members of which were affected by autosomal recessive STGD, were clinically examined. All 50 exons of the ABCR gene were screened by DHPLC and direct sequencing techniques.Results: In all patients we reported some mutations of ABCR gene . Some of them have been already described and among them Gly1961Glu was the most frequent in our series. Seven novel mutations were identified:three missense mutations (Thr970Pro, Phe1015Ile and Leu2221Pro); two nonsense mutations (Cys1177Stop and Trp1408Stop); two small deletions (5905delG and 6636delA). The missense mutations were not detected in 150 unaffected control individuals 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. The frequent report of novel mutations is probably related to the great number of exons in the ABCR gene, which favours rearrangements in DNA sequence.

Ref.Allikmets et al., 1997, Science, 227,1805.

P0677. Analysis of the expression levels of genes mapping in the critical region in WBS patients

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An increasing number of human diseases, such as the Williams-Beuren contiguous gene syndrome (WBS, OMIM#194050), are recognized to 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 often assumed that these aneuploidies will lead to underexpression of genes mapping to the commonly deleted region. But it is not clear that all genes will be expressed at half of the level observed in controls. For example, regulatory feedback loops may ensure that many genes are expressed at normal levels. In WBS patients no systematic attempt has been made to identify which genes are underexpressed, to what level, and, critically, in which tissues and at which time. Furthermore, it is conceivable that the large chromatin rearrangement harbored by WBS patients' influences the transcription levels of genes that map centromeric or telomeric to the critical region even if these genes are present in two copies. We have used quantitative real-time PCR to accurately measure the expression of the genes haploinsufficient in WBS patients. We studied in 25 WBS patients and 10 controls a total of 41 genes mapping to the WBS critical region, its flanking repeats and neighboring regions in two different cell lines (skin fibroblasts and lymphoblastoid). The measure of their relative expression will allow to assess the contribution of each genes to WBS features.

P0678. SLC22A5 homozygous 844delC mutation: sudden infant death and carnitine responsive cardiomyopathy in Roma families as novel phenotypes of the OCTN2 mutations

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We identified an inherited single base deletion of 844C of the SLC22A5 (solute carrier family 22 member 5) gene, causing a premature stop codon and thus a truncated protein in two non consanguineous Hungarian Roma (Gypsy) children presented with cardiomyopathy and slight myopathy. Introduction of carnitine therapy resulted in dramatic improvement of the cardiac symptoms. Family investigations revealed four sudden deaths, two of them corresponded to the classic SIDS phenotype. In postmortem tissue specimens we could verify the homozygous mutation. In liver specimens reserved from two patients lipid droplet vacuolization could be observed mainly in the peripherolobular regions of the acini. Besides a generalized hypertrophy, lipid vacuoles were also seen in the heart tissue, predominantly in the subendocardial areas. Review of all OCTN2 cases reported so far revealed that this is the first description of classic familial sudden infant death syndrome with definitively established SLC22A5 mutation and with presentation of the histopathology findings. Genetic analysis of 26 relatives with different parental lineages revealed 14 carriers; this strongly suggests a high prevalence of this mutation in certain Roma populations. Another novel feature of our cases is that the affected patients exhibited only decreased carnitine levels. This finding suggest that not only patients with zero or trace carnitine levels should be considered as carnitine deficiency.

P0679. Pathophysiology of syndromic combined pituitary hormone deficiency due to a LHX3 defect

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Combined pituitary hormone deficiency (CPHD) has been associated with mutations in genes encoding pituitary-specific transcription factors. This condition involves also extrapituitary abnormalities, thereby defining a syndromic form where both LHX3 and LHX4 (two closely related genes encoding LIM-homeodomain factors) are implicated; moreover, these two genes are believed to share redundant biological properties. The rare patients identified with a LHX3 mutation display a complete deficit in all but one anterior pituitary hormones, a short neck and anteverted shoulders. These features, not reported in Lhx3 and Lhx4 knock-out mice nor in patients with a LHX4 molecular defect, prompted us to analyze the molecular consequences of a LHX3 23-bp deletion involving a splice donor site identified in one family and the LHX3 and LHX4 expression patterns during early human development. This deletion leads to exon skipping, as revealed by in vitro LHX3 transcription. The resulting protein, which lacks the second LIM domain and the homeodomain, has lost transcriptional capability. Using in situ hybridization, we showed that the. LHX3 and LHX4 genes are expressed in the developing anterior pituitary gland and along the rostro-caudal length of the spinal cord. Here, they are mainly expressed in the ventral part giving rise to motorneurons and interneurons. Whereas LHX4 expression is transient, LHX3 is expressed at all stages studied. By contrast, no expression is detected in tissues forming muscles and the axial skeleton. This suggests that the extrapituitary anomalies result from a LHX3-dependent neurological defect. This defect is not rescued by the closely related protein LHX4.

P0680. Automated analysis of common mutations and polymorphisms in the human cystic fibrosis gene using a new diagmostic kit and Genemapper™ software

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Cystic Fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. We have developed a diagnostic device (kit) that 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. Separation (via capillary electrophoresis) and detection of the OLA fragments is achieved on the Applied Biosystems ABI PRISM® 3100 Genetic Analyzer where a full plate with 96 samples can be processed in under 4 hours of unattended operation. The resulting data are analyzed in an automated fashion using Applied Biosystems GeneMapper™ software that has been configured for CF. GeneMapper™ software presents genotype data along with quality control flags. It also allows for sorting of samples by the detected normal genotype or by heterozygous and homozygous mutations for final review by the investigator. We have validated the kit reagents and the CF-specific analysis system by correct detection and genotyping of all mutations present in the Coriell reference panel MUTCF, as well as mutations within a characterized set of clinical samples.

P0681. No mutation in the LMNA gene in four patients with Hallermann-Streiff syndrome

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Hallermann-Streiff syndrome is a rare inherited disorder characterized by small face with a thin pointed nose and microretrognathia, congenital cataracts, microphthalmia, hypotrichosis, skin atrophy, proportionate short stature, and neonatal teeth. Although the syndrome shares several phenotypic features both with progeria and mandibulofacial dysostosis, it is regarded a separate entity. We ascertained four patients presenting with typical signs of Hallermann-Streiff syndrome. Conventional cytogenetic analysis showed a normal karyotype in all patients. Although the pattern of inheritance is not yet known, autosomal dominant new mutations have been suggested. Recently, mutations in the *lamin A/C (LMNA)* gene have been found both in patients with progeria and mandibuloacral dysostosis. Due to the overlapping phenotypic features between progeria and Hallermann-Streiff syndrome, we speculated that the latter one might be an allelic disorder and performed mutation analysis of *LMNA*. In one patient, we identified a homozygous IVS6-44C>T transition that was also present in heterozygous state on one of 170 control alleles. A second patient was heterozygous for the mutation c.1930C>T (p.R644C). We found this heterozygous change in the mother of the patient suggesting that it was not associated with the disease. The same mutation was previously described in a patient with familial dilated cardiomyopathy, a disease known to be caused by heterozygous mutations in *LMNA*. However, the c.1930C>T change was also found in an unaffected individial. In summary, no obvious disease causing mutations were detected in the *LMNA* gene in four patients with Hallermann-Streiff syndrome suggesting that it is not the major gene for this syndrome.

P0682. Our first experience with establishment of the FASAY method for detection of loss-of-function mutations in the NF1 gene of the Czech patients.

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Neurofibromatosis type 1 (NF1) is one of the most frequent human genetic disorders, with an incidence of 1 in 3500. Associated with the abnormal growth of neural crest-derived cells, it is characterised by the presence of café-au-lait spots, cutaneous neurofibromas, plexiform neuromas, Lisch nodules. The NF1 gene is classified as tumour supressor gene. The gene product , neurofibromin, displays partial homology to GTPase-activating protein(GAP). The GAP-related domain (GRD), encoded by exons 20-27a, is the region with known biological function. The GRD of the human NF 1 protein (NF1GRD) can down-regulate RAS in Saccharomyces cerevisiae. We report our first experience with introducing of a technique termed the **FASAY method** (Functional Analysis of Separated Alleles in Yeast) for detection of heterozygous **NF1GRD** mutations in our NF1 patients. We established DNA/RNA banking of our clinicaly confirmed NF1 patients for retrospective or perspective study of this disease. To this date, our DNA/RNA bank consists of 150DNA samples and 40RNA samples of NFI patients. To optimalize the FASAY we start with transformation of E. coli (DH5α) by centromeric vector pRSPGK-NF1 (wild type) and pRSPGK (-NF1GRD) *kind gift of Dr. Chikashi Ishioka, Japan,* preparing linearized gap repair vector and mutant NF1GRD expression vectors as positive controls of FASAY analysis (QuikChange kit ,Stratagene)
We hope that this work with an introducing of the **functional analysis** of mutations in the extended NF1 GRD may well provide further insights into the mechanisms of pathogenesis associated with NF1.
This work is supported by the grant NM7627-3.

P0683. Novel compound heterozygote CYP1B1 mutations in a patient with Rieger's anomaly

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Glaucomas are a clinically and genetically heterogeneous group of optic neuropathies resulting in optic nerve atrophy which can lead to permanent loss of vision. The disease affects over 40 million people worldwide and is the second most prevalent cause of bilateral blindness in the Western world. Primary congenital glaucoma (PCG), associated with a primary angle defect, manifests at birth or in early childhood and follows an autosomal-recessive pattern of inheritance. Secondary congenital glaucoma is associated with a variety of anomalies involving different tissues of the eye summarized as anterior segment dysgenesis (ASD). Distinct subtypes of ASD include aniridia, Axenfeld's anomaly, Rieger's anomaly, iridogoniodysgenesis, Peters' anomaly and posterior embryotoxon. Mutations in the *CYP1B1* gene, a member of the cytochrome P450 superfamily, are frequently found in PCG patients but have also been reported for one Peters' anomaly patient. Mutations in *FOXC1*, a transcription factor, have been reported to cause Rieger- and Axenfeld's anomaly. We found two *CYP1B1* mutations (Trp57Stop, c.4832delCTC) in a sporadic patient with Rieger's anomaly and suspected congenital glaucoma. No family history of the disease was reported. The nonsense mutation Trp57Stop predicts a truncation of the P4501B1 polypeptide by 486 aminoacids. The c.4832delCTC mutation eliminates one out of three leucines, they are located in the highly conserved I-helix region of the protein. Sequence analysis of the entire coding region of the *FOXC1* gene revealed no mutation. This is the first report of *CYP1B1* mutations causing the complex symptoms of Rieger's anomaly.

P0684. Search for mutations causing non-syndromic X-linked mental retardation (NS-XLMR) by high-throughput PCR based sequencing and mRNA expression profiling of candidate genes on proximal Xp

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X-linked mental retardation (XLMR) affects approximately 2 out of 1000 males 2/3 of which show non-syndromic XLMR (NS-XLMR). Searching for disease-causing mutations in NS-XLMR is complicated by the genetic heterogeneity of this condition. However, by pooling weighted linkage data from 125 different families we could show that >30% of the mutations leading to XLMR are located on proximal

Xp and in the pericentric region (Ropers et al. 2003). Recently, we have screened 32 linked families for mutations in 48 brain-expressed genes from a 7.3Mb interval on Xp. In at least 10 of the families tested we have identified possibly causative mutations, which involve 5 different genes. To discover the causative mutations in the remaining 22 families, we are now investigating 28 additional genes, which had not been fully annotated at the start of the first screen or had been omitted for other reasons. We make use of a semi-automatic mutation screen consisting of automated primer design, PCR amplification, DHPLC and sequence analysis. As some disease-causing mutations may not be detectable by our PCR-based approach, we also screen mRNA from lymphoblastoid cell lines of patients for expression changes and altered splicing patterns. Until now, one third of 4202 PCR products have been analyzed, and 7 sequence variants in 4 different genes have been identified. Three of these variants were not found in healthy controls.

P0685. Identification of the molecular defects in Spanish and Argentinian mucopolysaccharidosis VI (Maroteaux-Lamy syndrome) patients, including 11 novel mutations

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Maroteaux-Lamy syndrome, or mucopolysaccharidosis VI (MPS VI) is an autosomal recessive lysosomal storage disorder due to the deficiency of N-acetylgalactosamine-4-sulfatase or arylsulfatase B (ARSB). Mutation analysis in 15 MPS VI Spanish and Argentinian patients resulted in the identification of 11 ARSB new mutant alleles: six missense mutations (p.L82A, p.Y138C, p.G308R, p.P313A, p.C447F and p.L472P); a nonsense mutation (p.W322X); a single nucleotide deletion (c.427delG); and three intronic changes affecting splice sites: IVS5-8(T>G) and IVS5-1(G>C), which disrupt the acceptor site of intron 5 resulting in skipping of exon 6, as shown by RT-PCR, and IVS5+2(T>A), not tested at RNA level. We also report seven previously described mutations (p.R95Q, p.R160Q, p.R160X, p.R315Q, c.238delG, c.237-243delGGTGCTC and a genomic deletion that includes exon 5), as well as several non pathogenic polymorphisms: IVS1-26(T>C), IVS5-27(A>C), p.G324G, p.V368M, p.V376M, p.S384N i p.P397P. Mutation analysis was performed by PCR amplification and sequencing of the ARSB eight exons and flanking regions. Molecular defects were confirmed by restriction analysis and have not been detected in 100 control chromosomes. This is the first report on ARSB mutations in Spanish and Argentinian populations. Most of the patients are compound heterozygotes, while only four of the patients are homozygous, demonstrating the broad allelic heterogeneity of the disease. Two mutations (IVS5-1 G>C and IVS5-8 T>G,) are present in both populations.

P0686. Mutational analysis of MYH9 in patients with giant platelet syndromes

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Autosomal dominant giant platelet syndromes including May Hegglin anomaly, Fechtner syndrome, Sebastian platelet syndrome and Alport like syndrome are considered monogenic disorders caused by mutations in *MYH9* encoding the heavy chain of non-muscle myosin IIA (NMMHC-IIA). By SSP or RFLP analysis, we have screened 18 patients suffering giant platelet syndrome for 16 known MYH9 mutations. In 12 patients we could identify the disorder causing mutation (amino acid changes; K371N, R702C, R702H, D1424H, D1424N (4 patients), E1841K (2 patients), and R1933X (2 patients)). Extending the mutational analysis to the whole gene by heteroduplex analysis and direct sequencing, we identified a novel DNA variation 172T>G leading to a predicted Y11X stop mutation resulting in a putatively truncated NMMHC-IIA protein. In the residual 5 patients no mutations in *MYH9* were found by heteroduplex analysis. As our study shows, it is necessary to screen the whole *MYH9* gene not just the known mutations. Possibly, only 72, 2 % of the patients suffering giant platelet syndromes are caused by mutations in *MYH9*. This leads to the conclusion that further so far unknown genes are associated to giant platelet syndromes.

P0687. Clinical, molecular, and genotype-phenotype coorelation study from 25 observations of oral-facial-digital syndrome type 1 : French and Belgian collaborative study.

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The oral-facial-digital type 1 is an X-linked dominant syndrome with lethality in males, belonging to the heterogeneous group of the oral-facial-digital syndromes. Clinical features include facial dysmorphism with hyperplastic buccal frenula, lingual hamartoma, bifid or lobulated tongue, cleft lip or palate, and distal anomalies such as brachydactyly. Polycystic kidney disease and central nervous system malformations are commonly associated. Inter- and intra-familial clinical variability is high. Recently, the OFD1 gene has been reported to Xp22.2-p22.3 and eighteen mutations have been reported in the literature. Here, we report on a French and Belgian collaborative study including 25 cases in 16 families. Clinical results were superimposable with those described in the literature except for previously unreported short stature (16 %). Direct sequencing analysis in the OFD1 gene identified 11 mutations, including 9 frameshift, 1 nonsense mutation and 1 missense mutation and spanning in 9 different exons. With the association to literature cases, we showed that majority of mutations (65.5 %) were located in exons 3, 8, 9, 13 and 16. Phenotype-genotype correlation performed on 46 cases revealed a significative correlation between polycystic kidneys disease and splice mutations versus other mutations, mental retardation and mutations located in exons 3, 8, 9, 13 and 16 versus other exons. X-inactivation study in lymphocytes was skewed in 6/19 cases and was of particular interest in three familial cases with striking intrafamilial variability. In conclusion, we suggest that clinical inter and intrafamilial variability could be explained at least in part by allelic heterogeneity and X-inactivation status.

P0688. Missense mutations and gene interruption in *PROSIT240*, a novel *TRAP240*-like gene, in patients with congenital heart defect (dTGA)

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Background - Congenital heart disease (CHD) represents the most common severe birth defect affecting 0.7-1% of all neonates, amongst which 5-7% display a transposition of the great arteries (TGA). TGA represents a septation defect of the common outflow tract of the heart, manifesting around the fifth week during embryonic development. Despite its high prevalence very little is known about the etiology of the disease.

Methods and Results - Using a positional cloning approach we isolated a novel gene, *PROSIT240*, that is interrupted in a patient with a chromosomal translocation, displaying a transposition of the great arteries and additionally mental retardation. High expression of *PROSIT240* within the heart (aorta) and the brain (cerebellum) correlates well with the malformations observed in the patient and prompted further analyses. *PROSIT240* shows significant homology to the nuclear receptor coactivator *TRAP240*, suggesting it to be a new component of the TRAP complex. Interestingly, several TRAP

components have been previously shown to be important in early embryonic development in various organisms, making *PROSIT240* an excellent candidate gene to be correlated to the patient´s phenotype. Subsequent mutational screening of 97 patients with isolated dTGA revealed three missense mutations in *PROSIT240*, which were not detected in 400 control chromosomes.
Conclusions - Together, these genetic data suggest that PROSIT240 is involved in early heart (and brain) development.
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P0689. Mutation analysis in Hereditary Angioedema identifies patients at risk for developing acute and life threatening edema

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Hereditary angioedema (HAE) is an autosomal dominant disease due to mutations of the C1 inhibitor gene (C1INH). The C1INH protein is the sole inhibitor of the C1r and C1s components of the classical complement pathway and the major regulator of factors XI and XII and of plasma kallikrein in the contact system.
HAE manifests as acute intermittent swellings of nearly every organ especially of the skin and the gastrointestinal tract. Laryngeal edema may cause suffocation and if not treated properly can be fatal.
We have sequenced the 8 exons of C1INH in 96 HAE families (157 members) from specialised outpatient clinics in Frankfurt and Mainz. In 20 families with 42 members, we found mutations that were already known from the literature. In 69 families with 103 members, we identified 55 different novel mutations. In further 7 families with 12 members, large deletions were detected by Southern blotting. The mutation spectrum is composed of 41% missense and 8% nonsense mutations, 31% small deletions, 10 % splice site mutations, 10% large deletions. This distribution corresponds well to published data. Our data bring to 174 the total number of C1INH mutations. Some of the novel missense mutations are pointing to functionally important amino acid residues within the C1 inhibitor protein.
Routine molecular genetic analysis by direct sequencing and Southern blotting is effective in identifying causative mutations in most HAE families. Early diagnosis of mutation carriers prior to clinical manifestation is essential in prevention and treatment of acute and life threatening edema.

P0690. The Bardet-Biedl syndrome: molecular analysis of 27 families

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Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder wich associates cognitive
impairement, retinitis pigmentosa, obesity, polydactyly, hypogenitalism and renal disease. The significant genetic heterogeneity in BBS is supported by the mapping of at least eight loci (BBS1 to 8). Among them, six genes are identified: BBS1, BBS2, BBS4, BBS6, BBS7 and BBS8. The BBS phenotype for some patients seems to require the presence of three mutant alleles at two different loci, defining “triallelic inheritance”. We report here the study of the six identified BBS genes in a cohort of 33 BBS patients from 27 families. Mutations have been scored in 14 families. Two mutations within the same gene have been identified in 7 families. BBS1 is most frequently implied with the common M390R substitution at the homozygous state (n=2), or associated with another mutation at BBS1 (n=3). Compound heterozygous mutations have been found in BBS2 (1 family), and BBS6 (1 family). In seven other families, only one heterozygous mutation has been identified (once in BBS1, twice for BBS2 and three times in BBS6). This high score of heterozygotes suggests the existence of at least another mutation, possibly at an unidentified gene. No patient was found to date to disclose triallelism. We currently extended our cohort of patients with 84 additional families, in order to further test the putative triallelic hypothesis and investigate other candidate genes.

P0691. FANCD2 as a key human caretaker gene with propensity to somatic reversion

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The conserved *FANCD2* gene serves as a central effector in the evolutionarily novel Fanconi anemia / BRCA caretaker pathway. Monoubiquitination of the FANCD2 protein appears crucial for efficient repair of DNA double strand breaks and interstrand crosslinks. Mutations in *FANCD2* result in the rare FA-D2 subtype of Fanconi anemia (FA): to date, only 8 of 241 FA patients in the European registry (EUFAR) have been assigned to this complementation group (LEVITUS et al., *Blood* online 2003). *FANCD2* mutation analysis is complicated by the presence of 8 pseudogene regions such that no *FANCD2* mutation have been published beyond those in the initial gene identification report (TIMMERS et al., *Mol Cell* 7, 249-262, 2001). Among FA LCL and primary fibroblast cultures that could not be assigned to any other complementation group, we identified by immunoblot and retroviral complementation screening 16 patients from 14 independent families as FA-D2. Sequence analysis of cDNA or/and gDNA from 7 patients revealed 1 homozygous missense mutation in a conserved region, 6 splice site mutations resulting in exon skipping, 2 small deletions, 1 frameshift insertion, and 1 nonsense mutation in the compound heterozygous patients. 5 patients developed mosaicism in hematopoetic precursor cells resulting in mitomycin C resistance in their lymphocytes, which hitherto has been described only for FA-A and FA-C patients. Our data classifies *FANCD2* as a FA gene with innate propensity to somatic reversion. This study contributes to an improved definition of the FA-D2 phenotype, the *FANCD2* mutation spectrum, and the somatic reversion mechanisms (“natural gene therapy”).

P0692. On the molecular basis of warfarin resistance in rats.

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For 60 years, coumarin drugs (e.g. warfarin) are in use for the treatment of thromboembolic events in humans and for pest control in rodents. Coumarins target blood coagulation via inhibition of the vitamin K epoxide reductase complex (VKOR), and in high doses lead to spontaneous fatal bleedings. VKOR recycles vitamin K 2,3-epoxide to vitamin K hydroquinone, an essential cofactor for the post-translational gamma-carboxylation of several blood coagulation factors.
Resistance to warfarin and its more potent derivatives (e.g. difenacoum, bromadiolon) has been reported for several wild populations of rats and mice. The recent identification of the VKORC1 gene, a key component of the VKOR, allowed for the characterisation of the molecular basis of warfarin resistance (Rw).
Four mutations in the rat VKORC1 gene have been identified in Rw animals from Denmark, Germany and the UK. While Danish and German rats share an Y139C mutation of supposedly common origin, three different mutations were observed in British rats all clustering in a hydrophobic domain of the protein. Upon recombinant expression, all Rw mutations conferred resistance to warfarin at the expense of a lower basal VKOR activity. This is in line with previous reports on

the reduced fitness of Rw rats in the absence of warfarin selection. VKORC1 mutations alone, however, do not explain the observed differences in sensitivity against second generation coumarins. Apparently, other modulating factors are required for multiple resistance.

P0693. A mutation creating an upstream initiation codon in the SOX9 5´ UTR causes campomelic dysplasia

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Campomelic dysplasia (CD) is a semilethal skeletal malformation syndrome that results from de novo heterozygous mutations in *SOX9*. These mutations are distributed over the entire coding region and cause loss-of-function of the protein, resulting in haploinsufficiency. We report on a 2 year-old girl with a 46,XX karyotype who has clinical and radiological features of surviving CD, including micrognathia, tracheomalacia, small scapulae, 11 pairs of ribs and short ischia. Sequence analysis of the *SOX9* coding region failed to reveal a mutation. However, a heterozygous mutation G>A at position 188 in the 5´ UTR was found. The patient´s mother, father and healthy brother were homozygous G/G at this position, as were 100 control chromosomes. Paternity was confirmed by microsatellite marker analysis. The G>A mutation creates an upstream translation start codon (GTG>ATG) with a much better fit of its flanking sequence to the Kozak consensus than that at the wildtype ATG start codon (4/6 vs. 1/6). If used, the upstream ATG would lead to translation of a short peptide of 62 amino acids from a reading frame that terminates just after the wildtype ATG start codon. Such an upstream open reading frame could reduce or prevent translation of the wildtype protein. Reduced amounts of SOX9 protein from the mutant mRNA would be compatible with the milder phenotype of the patient. Although described only rarely in human genetic disease and for the first time here for CD, mutations creating upstream ATG codons may be more common than generally assumed.

P0694. Influence of fibrillin-1 fragments on matrix metalloproteinase expression in cell culture: A potential factor in the pathogenesis of the Marfan syndrome

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The Marfan syndrome, a relatively common autosomal dominant disorder of connective tissue, is caused by mutations in the gene for fibrillin-1 (*FBN1*). Fibrillin-1 is the main component of the 10-12 nm microfibrils that together with elastin form elastic fibers found in tissues such as the aortic media. Recently, *FBN1* mutations have been shown to increase the susceptibility of fibrillin-1 to proteolysis *in vitro*, and other findings suggest that the upregulation of matrix metalloproteinases (MMPs) as well as fragmentation of microfibrils could play a role in the pathogenesis of the Marfan syndrome.
In the present work we have investigated the influence of fibrillin-1 fragments on the expression of MMP-1, MMP-2, and MMP-3 in a cell culture system. Cultured human dermal fibroblasts were incubated with several different recombinant fibrillin-1 fragments. The expression level of MMP-1, MMP-2, and MMP-3 was determined by quantitative RT-PCR, and the concentration of the corresponding proteins was estimated by quantitative Western blotting. Our results establish that treatment of cultured human dermal fibroblasts with several recombinant fibrillin-1 fragments induces up-regulation of MMP-1 and MMP-3. A similar effect was seen upon stimulation with a synthetic RGD peptide. The expression of MMP-2 was not influenced by treatment.
Our results suggest that fibrillin fragments may themselves have pathogenic effects by leading to upregulation of MMPs, which in turn may play a part in the progressive breakdown of microfibrils thought to play a role in Marfan syndrome.

P0695. Description of a CDKN1C mutation in familial BWS with variable penetrance

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BWS is a congenital overgrowth syndrome with an increased risk of developing embryonic tumours, such as Wilms` tumour (WT) (5% of affected children). The cardinal features are exomphalos, macroglossia and neonate gigantism. BWS is generally sporadic, only 10-15% have a familial history. To date, no general molecular basis for BWS has been defined. The only mutations identified, are loss-of-function mutations in the imprinted CDKN1C gene. Such mutations appear to be associated with increased umbilical abnormalities, but not with increased WT predisposition. In the remaining BWS subgroups an alteration of the tight epigenetic regulation of 11p gene expression (e.g. patUPD) seems to be the cause of the syndrome. We analysed three generations of a family with a mother and one child affected with cardinal features of BWS (without WT). After excluding a patUPD and hypomethylation at KCNQ1OT1, we sequenced the CDKN1C gene and found a 16 bp deletion in exon 2 at position 1977-1992 (C1977del16). Interestingly this missense mutation was heterozygously present in the affected persons as well as in the maternal grandmother, who did not suffer from BWS. The preferential maternal expression of CDKN1C goes along with the maternal transmission of the phenotype and may explain why the grandmother is not affected. One remarkable observation is that the mother has only mild BWS characteristics. Familial BWS cases with variable phenotypes enable the determination and a differential analysis of effector genes. Prospects in this regard are discussed.

P0696. Characterization of deletions in patients with spinal muscular atrophy (SMA) type I,II and III and determination of SMA carriers using MLPA analysis

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In about 95% of patients with spinal muscular atrophy (SMA) mutations have been identified in the SMN1 gene localized at chromosome 5q12-13.The clinical phenotype of SMA varies from the lethal SMA type I, the intermediate type II and the milder type III. Almost 95% of patients of all three clinical types have a homozygous deletion of at least exon 7 of the SMN1 gene. A very similar gene, SMN2, centromeric to SMN1, has been proposed as a modifying gene, since patients with the milder forms of SMA appear to have more SMN2 gene copies. A novel multiplex ligation-dependent probe (MLPA) assay has been adapted by us to quantify SMN1 and SMN2 gene copy numbers. SMA carrier analysis using MLPA has been validated by comparison with results obtained by our conventional dosimetric exon 7 SMN analysis of 54 SMA carriers and 49 normal control samples. MLPA analysis did not result in any discrepancies. Subsequently, a total of 19 SMA type I, 30 type II and 27 type III patients were tested and in all these cases at least exon 7 of SMN1 was absent. The MLPA can be used as a robust and reliable method for diagnosis of SMA patients and carriers. Upon MLPA analysis about 84% of type I, 93% of type II and 81% of SMA type III patients carried a total of 2, 3 and 4 SMN2 gene copies, respectively, confirming that the SMA phenotype is clearly associated with the number of SMN2 gene copies.

P0697. How to prepare stable reference materials for genetic testing

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In order to harmonise, standardise and improve the quality of genetic diagnostic services the establishment of reference measurement systems is of pivotal importance. Diagnostic measurements must

be accurate, precise, specific, comparable among laboratories and traceable to the available reference measurement procedures and available reference materials of a higher order. CRMs are recognised as excellent means to check analytical accuracy and create crucial reference points in the process of the development of comprehensive measurement systems. The current lack of CRMs for molecular genetic tests leads to assays not traceable to an accepted common standard.
A number of genetic CRMs are presently under development by a European Consortium including Institute for Reference Materials and Measurements (IRMM) in the course of the ongoing CRMGEN shared cost action project funded by the EC, and by IRMM in close co-operation with the Committee of Molecular Biology Techniques of the IFCC.
Stability of the produced DNA-based CRMs is of central importance. In order to improve the stability of genetic CRMs and optimise the DNA preservation,
-Testing protocols were optimised and described according to ISO GUIDE 34 and BCR guidelines,
- A model system for certification of DNA-based RMs was established,
- Different approaches for stabilisation of nucleic acid-based RMs were investigated,
- Several additives were tested to achieve high stability,
- Freeze-drying processing and packaging were optimised to improve the stability and recovery rate.
The knowledge gained in this feasibility study will be used to produce stable and suitable and commutable CRMs for clinical genetic testing.

P0698. Certified Reference Materials (CRMs) for the analysis of the human factor II (prothrombin) gene G20210A mutation

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The recent progress in molecular pathology made DNA-based assays an important area of laboratory medicine and essential in patient care. DNA tests allow the detection of genetic alterations responsible for inheritable disease conditions, higher risk to develop diseases or altered drug metabolism.
Although DNA tests have a high impact on the clinical decision-making, and the number of tests performed in diagnostic laboratories is high, issues of quality have not yet been given sufficient attention, and currently no CRMs for clinical genetic testing available.
Therefore, the Scientific Committee of Molecular Biology Techniques (C-MBT) in Clinical Chemistry of the IFCC has initiated a joint project in co-operation with the European Commission- Joint Research Centre, Institute of Reference Materials and Measurements to develop and produce plasmidic CRMs for the analysis of the human prothrombin gene G20210A mutation.
A gene fragment chosen was produced that spans all primer annealing sites published until today. Both the wildtype and the mutated alleles of this gene fragment were cloned into a pUC18 plasmid and two plasmidic RMs were produced. In addition a mixture of both plasmids was produced mimicking the heterozygous situation.
In the present study, different possibilities to stabilise and package purified DNA were also investigated to improve stability and recovery rate of the CRMs after freeze-drying. To assess the commutability of these reference materials, a field study was launched, where the material performed with excellence.
This series of plasmidic CRMs are to the best of our knowledge the world-wide first clinical genetic CRMs introduced.

P0699. Effect of polyglutamine expansion on gene expression in SCA3.

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Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the *MJD1* gene encoding a polyglutamine repeat in the respective ataxin-3 protein. The reason why expanded polyglutamines lead to neurodegeneration in SCA3 patients remains uncertain to a great extend.
In order to analyse the effect of polyglutamine expansions in the ataxin-3 protein on gene expression patterns we generated three stable transfected human SK-N-AS neuroblastoma cell lines expressing ataxin-3 with a variable number of polyglutamine repeats: 15 polyglutamine repeats as control as well as 77, and 148 repeats as disease models, respectively. A comparable expression of the introduced ataxin-3 construct among the different cell lines was confirmed by western blotting. For the expression analysis total RNA was isolated from three independent cultures of each cell line grown under identical conditions. Differences in gene expression would therefore be solely due to different numbers of polyglutamine repeats within the ataxin-3 protein. The analysis of gene expression was carried out using the Human Genome U133 Plus 2.0 Array (Affymetrix) covering about 47.000 different transcripts. The optained microarray data was analysed by pairwise comparison of the three disease model samples with the controls. Genes with uniform regulation in seven of these nine comparisons were defined as differently expressed genes. We identified in our study genes out of twelve functional groups which are differentially expressed as a result of a polyglutamine expansion in the ataxin-3 protein.

P0700. Development and application of a full-coverage X-chromosomal BAC array for high-throughput screening of genomic alterations in patients with X-linked mental retardation.

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Current genetic and molecular data suggest that up to 100 genes for nonspeficic X-linked mental retardation (MRX) exist. About 15 of these have been identified. Seven of these 15 MRX genes have been identified upon characterisation of chromosomal aberrations, such as translocations and deletions. Since most of the cytogenetically visible X-chromosomal deletions have already been studied, novel methods are needed to efficiently identify small submicroscopic alterations in MRX patients. The technology that we have focussed on is array-based comparative genomic hybridisation (arrayCGH). We have constructed a full-coverage X-chromosomal genomic BAC array consisting of approximately 1500 clones. The sensitivity and specificity of the technology were tested in a series of normal versus normal control experiments and a series of patients with known copy number changes at the X chromosome. Our array allows the detection of copy number changes smaller than 100 kb on the human X chromosome. Next, the full-coverage chromosome X-array was used for the analysis of selected MRX patients from the European XLMR consortium. In the first set of 24 linked families we already have identified several single copy number changes, which had remained uncovered upon traditional karyotyping and previous linkage analyses. Both deletions and duplications were detected, ranging in size from 200 kb to 7 Mb. This study clearly demonstrates the power of the arrayCGH technology. Deletion and amplification mapping can now be performed at the submicroscopic level and will allow high throughput identification of chromosomal regions harboring MRX genes.

P0701. Two cases of alobar holoprosencephaly and mild facial dysmorphism with novel *ZIC2* mutations.

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Holoprosencephaly (HPE) is a brain malformation characterised by failure of proper formation of midline structures of the forebrain. It is relatively common, with an incidence of approximately 1 in 16000 live births and 1 in 250 during embryonic development. The aetiology of HPE is heterogeneous and includes chromosomal aberrations, teratogenic factors and single gene defects.
We report on two cases of HPE. Both patients showed alobar HPE with a large monoventricle and lack of forebrain midline structures. In contrast to the severe brain malformation, both patients showed only mild facial dysmorphism with mild hypotelorism and midface hypoplasia. Both developed macrocephaly and died at the age of 8 years (case 1) and 3 months (case 2), respectively.
In both cases we identified mutations in exon 1 of the *ZIC2* gene. Case 1 had a heterozygous deletion c.661delA resulting in a frameshift and a premature termination codon (N221fsX224). In case 2 we identified a heterozygous nonsense mutation c.782G>A, leading to a stop codon (W261X).
Our report expands the number of reported *ZIC2* mutations in holoprosencephaly to 13, including the first nonsense mutation. Of all cases of *ZIC2* mutations reported so far, no patient showed severe facial dysmorphism despite the frequently observed severe brain phenotype. Therefore, the detection rate of mutations in *ZIC2* might be considerably higher in the subgroup of patients with alobar holoprosencephaly without severe facial dysmorphisms as compared to the detection rate of 3-4% for *ZIC2* mutations in the overall group of sporadic HPE cases.

P0702. The Mouse Mutant „Short Digits“ (Dsh) a Model for the Regulation of Sonic Hedgehog and Brachydactyly

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The mouse mutant „short digits“ (Dsh) is a radiation induced mutant with a complete holoprosencephaly phenotype in the homozygote and a limb reduction phenotype in the heterozygote. Analyzing 950 meioses Dsh/+ was fine mapped within a 12 MB interval on mouse chromosome 5, comprising Sonic Hedgehog (Shh). Shh expression is severly downregulated in the homozygote, yet no mutation was detected within the Shh coding region or conserved putative enhancer elements. Using a complementation assay we show that Dsh and Shh-/- are allelic. The heterozygous Dsh mutant exhibits missing/fused second phalanges of all digits and missing interphalangeal joints reminiscent of human brachydactyly type A1. Histological analysis reveals disruption of chondrogenesis and joint formation starting from E13.5 in Dsh/+. In the developing limbs of Dsh/+ the expression patterns of Ihh, Ptc and Gli genes as well as Gdf5 and Pthlh are disrupted starting at E13.5. The presented data make it highly likely that in the Dsh mutant a genomic inversion disrupts Shh regulation by numerous conserved long range cis-enhancer elements that reside outside the mapping interval. The heterozygous phenotype may be explained by disruption of Ihh and Gdf5 signaling in the heterozygote possibly through alteration of Shh regulation of a higly conserved limb enhancer located approximately 1 Megabase upstream of Shh.

P0703. XY sex reversal, caused by inactivation of one SOX9 allele in humans, needs inactivation of both Sox9 alleles in mice

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Heterozygous inactivating mutations in SOX9 cause the human skeletal malformation syndrome campomelic dysplasia (CD) together with XY sex reversal. In contrast, heterozygous Sox9 mutant mice do not show any XY sex reversal. Homozygous Sox9 null mutant mice die before E11.5, preventing the study of gonadal development. In mammals, SOX9 is expressed in the undifferentiated gonad of both sexes just before the onset of male sex determination. This expression continues in Sertoli cells during the entire testis development, whereas in developing female gonads, Sox9 expression is downregulated after E11.5. In this study, we have homozygously inactivated Sox9 during mouse gonadal development using the Cre/loxP system. XY mutant gonads develop as ovotestes or ovaries. In mutant ovaries, at E13.5, expression of Sox9 and of other early testis markers such as Amh and Sf1 were not detected; at E14.5, Dax1, a gene with differential sexual expression, resembles the female expression pattern, and Dmc1, an early ovarian meiosis marker, is detected. In sex-reversed mice with complete absence of testis cords, sexual ducts develop along the female pathway. We conclude that during gonadal development, Sox9 is necessary for testis cord formation and for the expression of early male pathway genes, and that, other than in humans, inactivation of both Sox9 alleles is needed to cause XY sex reversal.

P0704. Expression analysis of mutant alleles identified in Gaucher disease patients revealed possible “modifier” polymorphisms

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Gaucher disease, the most prevalent sphingolipidosis, is caused by the deficient activity of acid beta-glucosidase, mainly due to mutations in the GBA gene. Over 200 mutations have been identified worldwide, more than 25 of which were in Spanish patients. In order to demonstrate causality for Gaucher disease, some of them: p.P182L, p.N188S, p.R257X, p.Y313H, p.E326K, p.P391L, p.N392I, p.I402T and the double mutants [p.N188S; p.E326K] and [p.L444P; p.E326K], were expressed in Sf9 cells using a baculovirus expression system. Besides, mutations p.N370S, p.D409H and p.L444P were also expressed for comparison. The levels of residual acid beta-glucosidase activity for some of the mutant enzymes were negligible. Other group of enzymes (p.N370S, p.I402T, p.D409H, p.L444P and [p.L444P; p.E326K]) gave levels of activity ranging from 6% to 14% of the wild type. The mutant enzymes produced by the cDNAs carrying alleles p.N188S and p.E326K showed high activity (66.6% and 42.7% of wild type enzyme, respectively). Expression studies revealed that the p.E326K change, which was never found alone in a Gaucher disease-causing allele, when found in a double mutant such as [p.N188S; p.E326K] and [p.L444P; p.E326K], decreases activity compared with that found for the other mutation alone. These results suggest that p.E326K should be considered a “modifier variant” rather than a neutral polymorphism, as previously considered. Mutation p.N188S, which produces a mutant enzyme with the highest level of activity, is probably a very mild mutation or another “modifier variant”.

P0705. New mutations of the *NF1* gene on patients with neurofibromatosis type 1.

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder affecting ~ 1 in 3500 individuals. NF1 is fully penetrant at the age of 5 years and has a variable clinical expression, even among members of the same family. High number of sporadic cases (up to 50%) is observed. The disorder is caused by mutations in the *NF1* gene, one of the largest human genes, composed of 60 exons and spanning more than 300 kb of genomic DNA, and is considered to be a tumor-suppressor gene. It has a high mutation rate with most mutations predicted to lead to truncated proteins. The mutations occur throughout the gene and most have a frequency of less than 1%. A comprehensive analysis of the mutational spectrum might help to elucidate the role of different *NF1* mutations. DNA from periferal blood leucocytes of 25 patients (7 familial and 18 sporadic cases) with NF1 were screened for mutations. For each patient the whole coding sequence and adjacent intronic sequenses were investigated. We have used SSCP-method with subsequent direct sequencing of abnormal bands. Three novel mutations were found: six-nucleotide in-frame deletion in exon 28 (4973-4978delTCTATA), deletion A in exon 29 (position 5248), resulting in frameshift and T→A substitution in exon 29 (position 5498). We are currently working on development of indirect DNA methods for prenatal diagnosis in NF1 families and

on optimizing the effectiveness of mutation screening.

P0706. Novel Lamin A/C mutation in dilated cardiomyopathy patient

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Dilated cardiomyopathy (DCM) is a myocardial disorder characterized by ventricular dilatation and impaired systolic function leading to heart failure and sudden death. About one-third of idiopathic DCM are inherited, autosomal dominant form occurs most frequently and exhibits both clinical variability and genetic heterogeneity. To date 11 genes, including lamin A/C (LMNA) gene, have been associated with autosomal dominant DCM.
Here we described patient with diagnosis DCM confirmed at 6 month who had prenatal hypotrophy, heart failure, acute dilatation of left ventriculum and atrium and mitral regurgitation, ejection fraction 29%. Proband's parents were healthy. We have studied DNA of this patient by PCR-SSCP analysis of LMNA gene coding region (12 exons) divided into 14 fragments with sequencing of abnormal conforms. Gly635Asp mutation was found in exon 11. This mutation was not described previously.
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P0707. Transcriptional alterations and retinal degeneration in SCA7 and HD transgenic mice

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Huntington's disease (HD) and Spinocerebellar Ataxia type7 (SCA7) belong to a group of nine inherited neurodegenerative diseases caused by a polyglutamine expansion in the respective proteins. Regional neurodegeneration is specific to each disease, in particular SCA7 is the only one to display degeneration in the retina, a tissue usually spared in HD. Recent evidences indicate that transcriptional alterations could underlie the retinal dysfunction in SCA7. We previously showed that a SCA7 mouse model (R7E) and the R62 HD mice develop a similar retinal phenotype as revealed by progressive thinning of photoreceptors segments and decreased rod ERG response. Comparison of retinopathy in HD and SCA7 mice offers the opportunity to assess whether the molecular pathways underlying rod dysfunction are identical. Here we report a gene expression analysis on R7E, R6 and control retina using MOE 430A Affymetrix Genechips (22 690 probe sets). Around 11500 transcripts were detected present in the retina of control mice. We observed in both mouse models a downregulation of several components of the phototransduction pathway. These results were confirmed by RT-PCR and Northern blot experiments, showing an early, and drastic downregulation of rhodopsin suggesting that downregulation of photoreceptor genes expression plays a critical role in polyglutamine induced retinal degeneration. Down and up regulation of genes involved in other pathways was also observed. A similar number of genes was found dysregulated between the R6 and the R7E mice.

P0708. Molecular-genetic study of LMNA gene in Russian Emery-Dreifuss muscular dystrophy patients.

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Emery-Dreifuss muscular dystrophy (EDMD) is inherited disorder characterized by early contractures of elbows and Achilles tendons, slowly progressive muscle wasting and weakness, and a cardiomyopathy with conduction blocks that is life-threatening. Three modes of inheritance exist: X-linked, autosomal dominant, and autosomal recessive. Autosomal forms of Emery-Dreifuss muscular dystrophy (EDMD) are caused by mutations in the gene encoding lamin A/C (LMNA; 150330).
We have studied DNA of 22 EDMD patients by PCR-SSCP analysis of LMNA gene coding region (12 exons) divided into 14 fragments with sequencing of fragments revealed abnormal patterns. Two novel mutations were identified: Asp47His in exon 1 and Gly232Arg in exon 4. No one known mutation was found. Also we have found following polymorphisms in affected patients: T1073C, ivs5-22g→a, ivs5-25g→c, ivs5-30c→a, ivs5-31g→c in exon 5; ivs8-44c→t in exon 9; C1910T in exon 10; G2286C in exon 12 with frequecies 4,5%, 9%, 9%, 9%, 4,5%, 18%, 95%, 9% per chromosom respectively.
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P0709. Real-time quantitative PCR as a routine method for screening large rearrangements in Rett Syndrome: report of three cases of MECP2 deletion and one case of MECP2 duplication.

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Mutations in the X-linked MECP2 gene are found in 70-80 % of cases of classical Rett syndrome (RTT) and in about 50% of cases of Preserved Speech Variant (PSV). This high percentage of MECP2 mutations, especially in classical RTT cases, suggests that another major RTT locus is unlikely. Missed mutations may be due to the limited sensitivity of the methodology used for mutation scanning and/or the presence of intronic mutations. In a double copy gene, such as MECP2 in females, current methodologies (DGGE, SSCP, DHPLC, direct sequencing) are prone to miss gross rearrangements. Three previous reports have shown the presence of large deletions in a fraction of MECP2-negative classical RTT patients. We developed a reliable, single tube, quantitative PCR assay for rapid determination of MECP2 gene dosage. This method involves a multiplex reaction using a FAM labeled TaqMan probe with a TAMRA quencher derived from MECP2 exon 4 and two primers derived from the same exon and RNAaseP as an internal reference. We validated this assay through the analysis of 15 female and 15 male healthy controls and we then applied this method to 20 classic RTT and 12 PSV patients, all negative for MECP2 mutations. We identified four gross rearrangements: three deletions in classical RTT patients and one duplication in a PSV patient. Our results confirm that a fraction of MECP2-negative RTT cases have MECP2 gross rearrangements and we propose real-time quantitative PCR as a simple and reliable method for routine screening of MECP2 in addition to DHPLC analysis.

P0710. Germ-line mosaicism in Rett syndrome: is it more common than expected ?

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Rett syndrome is an X-linked neurodevelopmental dominant disorder that almost exclusively affects girls. The vast majority of cases are sporadic and are caused by de novo mutations in MECP2 gene, located in Xq28. Only a few familial cases have been reported: in four cases the mother was an asymptomatic carrier and in two cases the germ-line mosaicism in the mother was postulated. Due to the above reported cases of germ-line mosaicism we decided to offer prenatal diagnosis to all expectant mothers with a Rett daughter despite the absence of the causative mutation in their blood. We describe here the outcome of the first eight cases of prenatal diagnosis carried out by our center. In seven cases (5 females and 2 males) the fetus did not carry the mutation. In one case the female fetus did carry the same mutation as the affected sister. The couple decided to interrupt the pregnancy and to devolve fetal tissues of the affected fetus for research purposes. This positive prenatal test strengthens the importance of performing prenatal diagnosis in all cases of Rett syndrome, even when the mutation is apparently de novo. Moreover, our results indicate that germ-line mosaicism may be considered for the assessment of recurrence risk during genetic counseling.

P0711. Increased PARP-1 (ADP ribosylation 1) in lymphocytes from elderly subjects and premature aging syndromes

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Natural aging process is associated with decreased efficiency of DNA repair mechanism and oxidative stress mediated DNA damages. Damaging oxidative stress includes reactive oxygen species (ROS) generating chemicals, UV radiation and ionizing radiation. High permanent concentration of ROS induce a variety of damages in DNA including oxidized bases, DNA strand breaks, formation of cross links between DNA and proteins.
The DNA fragmentation is to be known to induce poly-ADP ribosylation which is a posttranslational modification of nuclear proteins,catalyzed by poly (ADP- Ribose) polymerase (PARP-1), known to be involved in in cell proliferation, differentiation and transformation as well as in recovery from DNA damage. Activation PARP-1 is one of the earliest respons of mammalian cells to DNA damage. Therefore detection PARP-1 level will confirm the presentation of actuel DNA strand breaks.
There are few studies about poly (ADP-ribosylation) activation regarding natural aging but none about premature aging so far. For searching the relationship with aging and PARP-1 and DNA fargmentation; we carried out a comperative study on lymphocytes by natural elderly subjects, premature aging syndrome patients and healthy young adults as a control group.
As a result there was significant increase in PARP activity in lymphocytes from Hutchinson-Gilford progeria and Werner syndrome patients when cmpoared with the elderly subjects and healthy young controls. Highest level were detected in progeria syndromes. DNA fragmentations were also significantly increased by both aged groups. The results show that, the increase rate of poly (ADP-ribosylation) in lymphocytes correlates interestingly with the speed of the aging process.

P0712. Mutation analysis in 202 tuberous sclerosis patients using a combination of different methodical approaches

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Tuberous sclerosis is an autosomal dominant disorder caused by mutations in TSC1 or TSC2. Worldwide efforts in mutation detection demonstrated a broad spectrum of lesions in both genes without mutational hotspots. In our study, 202 patients with a definite or suspected clinical diagnosis of TSC have been referred to genetic testing. In the first 104 patients, the Protein Truncation Test was applied for the TSC1 and TSC2 coding regions and supplemented with a screening of mutation cluster regions in TSC1 by HDA/TGGE and in TSC2 by DHPLC. Southern Blot analysis was used for large TSC2 deletion screening in all 104 cases. In a second approach, 98 additional patients were analysed by direct sequencing of all 62 exons of both genes. Patients tested negative were subsequently screened for large TSC2 deletions by FISH and long range PCR. Mutations were identified in 133 families. Detection rates were 66% for PTT combined with HDA/TGGE, DHPLC and Southern Blot in the first 104 patients compared to 65% for sequencing followed by FISH and long range PCR in the second 98 cases. Truncating TSC1 mutations were identified in 32 cases including 14 families. Of the 101 detected TSC2 mutations, 72 are small truncating, 6 large deletions, 20 missense mutations and 3 one-amino acid deletions. Of 13 TSC2 families identified by genetic testing, only 7 had previously been known as familial cases. The remaining 6 have been discovered by the evaluation of family members of index patients with unclassified missense mutations or small in frame deletions.

P0713. Genetic analysis of extra- and intragenic marker haplotypes in a group of cystic fibrosis patients from Serbia and Montenegro

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Cystic fibrosis (CF) is the most common fatal autosome recessive disease in Caucasians. Worldwide collaboration has resulted in discovery of almost 1300 mutations in CFTR gene and a large number of polymorphisms.
After screening of 175 CF patients from Serbia and Montenegro, we have detected 18 different CFTR mutations with F508del being the most frequent (72.28% of CF alleles). A total of 18 mutations cover 82.57% of CF alleles in this group.
Since the main goal of diagnostic analysis was to allow families at risk to do prenatal diagnosis, we have started with haplotype analysis in all cases where one or both CF alleles remain uncharacterized. Analysis was done for 6 diallelic sites and one tetranucleotide repeat (XV2C-KM19-MP6D9-J44-IVS6a(GATT)-M470V-T854T) on 58 F508del, 11 non-F508del and 40 normal chromosomes. The GATT polymorphism was detected by difference in mobility of the PCR product on 8% PAGE gel. Other haplotypes were determined after restriction digests of the RFLP-PCR products were separated by agarose gel electrophoresis. Strong linkeage disequilibrium was observed for CFTR mutations and one haplotype (1-2-2-1-6-1-1), while normal chromosomes mostly were associated with another one (2-1-1-1-7-1-2).
In this work authors will discuss their results in details, showing the importance of haplotype analysis in population where the molecular basis of CF is highly heterogeneous.

P0714. Heterozygous PITX2/RIEG1 whole gene deletion is associated with the Growth Hormone deficiency of the Rieger syndrome

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Background: Rieger syndrome(RS)is a developmental disorder, which associates Rieger ocular anomaly , umbilical defects and hypodontia. Additional features have been reported in RS patients and most notably growth hormone deficiency. None of these patients were found to have mutations in the PITX2/RIEG1 gene contrarily to 40% of our patients with isolated Rieger syndrome .
Purpose: We hypothesised that the RS patients with GH deficiency may have deletion of the PITX2 gene a midline pituitary expressed bicoid transcription factor gene.
Methods : We tested 4 families with RS plus GH deficiency or short stature by FISH using 4 cosmids containing the PITX2 gene and microsatellite markers form the region.
Results: We found 2 families with heterozygous deletion of the PITX2 gene using FISH and microsatellites. This confirms the major role of PITX2 as a midline and pituitary expressed gene although it is still unclear whether the PITX2 deletion alone is sufficient to cause the GH deficiency.

P0715. Scad Deficiency: Approach To A Diagnostic Challenge

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The clinical phenotype of short-chain acyl-CoA dehydrogenase (SCAD) deficiency is highly variable in age of onset and severity of symptoms. Similarly, ethylmalonic aciduria (EMA) and elevations of C4-acylcarnitine (C4) in blood are informative but not specific markers. The existence of possibly pathogenic SCAD gene variants and the absence of reliable enzyme assays in tissue other than muscle make the diagnosis of SCAD deficiency a significant diagnostic challenge.
We report our experience with a tiered approach to patients evaluated because of abnormal newborn (n=7) or high risk screening (EMA and/or C4) results (n=38). Methods: Plasma and urine were analyzed for acylcarnitines and organic acids, respectively. When the EMA excretion and/or C4 concentration was elevated, fibroblast

cultures were established for in vitro probing with L-carnitine, palmitic acid, and labeled L-valine and L-isoleucine followed by acylcarnitine analysis of the culture medium using tandem mass spectrometry. Sequencing of the SCAD gene followed when indicated. Results: 29 of 45 (64%) patient cell lines accumulated butyrylcarnitine in vitro (0.26-2.04 µmol/g prot.; controls: <0.25). Six carried 2 pathogenic mutations. Ten carried 1 pathogenic mutation in addition to homozygosity (4/10) or heterozygosity (6/10) for the common variants. Two cases were diagnosed with isobutyryl-CoA dehydrogenase (IBD) deficiency and 2 with deficiency of complex I of the respiratory chain by another method. Conclusion: This diagnostic approach reliably identifies patients with SCAD and IBD deficiencies. For patients with either normal or only mildly abnormal butyrylcarnitine levels in fibroblasts and absence of pathogenic mutations alternative diagnoses should be pursued.

P0716. Molecular analysis of haemophilia B in Iranian patients: three new mutations in the FIX gene.

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Haemophilia B is a X-linked recessive bleeding disorder caused by a deficiency of factor IX affecting 1/30 000 males. There are about 800 haemophilia B patients in Iran and the identification of the causative mutation in the factor IX genes of these patients and their families leads to precise carrier detection and prenatal diagnosis in affected families. DNA samples were obtained from 29 unrelated haemophilia B patients and some of their female relatives. The whole of FIX gene sequence, including intron/exon flanking regions and promoter, was analysed by conformation sensitive gel electrophoresis (CSGE). Only the exons with abnormal band pattern were sequenced and a total of 25 mutations were identified. However in four of them, no abnormal band was detected by CSGE. For these patients, all the exons and flanking regions have been sequenced and a mutation was found in all 4 patients. The identified mutations included 28 base substitutions and an isolated one base deletion. Three of these mutations (31267deltaA, G93S, Q246K) have not been previously reported, but the others have been previously reported and present in the database. It was interesting to note that more than 85% of theses mutations have been found in exon 2, 5 and 8 (30%, 15% and 42% respectively) suggesting that mutations in these 3 exons are very frequent in our haemophilia B patients. Analysis of factor IX mutation in the rest of our haemophilia B patients is now in progress for precise carrier detection and possible use in prenatal diagnosis.

P0717. Genetic testing and counseling of FMF patients in Armenia

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Molecular analysis of 3000 patients with Familial Mediterranean Fever (FMF, MIM 249100) demonstrated correlation between spectrum of MEFV mutations and clinical severity, including renal amyloidosis. Carriers of mutations in 250 healthy persons were 1: 5 with distribution of most mutations: M694V (4.7%); V726A (4.6%); M680I (1.8%); R761H (0.2%); F479L (0.4%); P369S (4.9%); E148Q (3.4%) was different from data obtained from patients: M694V (50.6%), V726A (22.3%), M680I (18.7%), R761H (3.2%), M694I (0.4%); E148Q (2.2%), F479L (1.3%). 98.65% of patients had 7 mutations, and in 77% both alleles were mutated in exon 10. Most part carried two mutations (74.8%) compared with patients with one (18.6%), three (0.7%) and without mutations (5.9%). Frequent genotypes were M694V/M694V (20.9%), M694V/V726A (18%), M694V/M680I (12.7%), M680I/V726A (9.8%), M680I/M680I (3.4%), V726A/V726A (2.8%), M694V/R761H (2.8%). Differences between frequency of mutations among patients and healthy individuals suggests that E148Q mutation was associated with a mild phenotype, and P369S mutation was found in asymptomatic carriers. Genotypes with P369S mutation were not associated with clinical features. M694V homozygous genotype had an unfavorable prognostic value for renal amyloidosis. Results suggest that penetrance depends on type of mutations involved in pathogenesis of FMF. Pedigree analysis of 200 families suggests that parent-to-offspring transmission of mutations was autosomal recessive in 91.5% and pseudo-dominant in 8.49%.

P0718. The DNA-diagnostics of X-linked recessive Emery-Dreifuss muscular dystrophy

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Emery-Dreifuss muscular dystrophy (EDMD) is characterized by early contractures, slowly progressive muscle wasting and weakness with a distinctive humero-peroneal distribution and cardiac conduction defects leading to dilated cardiomyopathy. There are autosomal and X-linked forms of EDMD which connected with mutations in lamin A/C gene (1q21.2) and mutations in emerin gene (EMD), which is located in Xq28 respectively. For DNA diagnostics of X-linked EDMD we have carried out PCR-SSCP analysis all EMD 6 exons in 23 male patients. We have reviled the alteration of electrophoretic mobility in some exons in fore different patients. During sequence analysis we determined two point mutations, one of them (Tyr34Stop) was known and published in EMD database and other (Asp72Val) was unknown previously. The next novel mutation is 2- nucleotides deletion in combination with 12- nucleotides insertion after nt72, which result in frame-shift in EMD first exon. The last reviled mutation is 4 bp insertion after nt 2294 in second exon EMD which result in frame-shift too. This mutation also is unpublished previously. More severe clinical features including cardiomyopathy appeared in patient with frame-shift mutation in first exon EMD.

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P0719. The spectrum of mutations in CMT1X patients from Russia

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We have been studying Russia Charcot-Marie-Tooth neuropathy (CMT) type 1X patients for the last several years. At present, our family material consists of 31 families, of which 104 patients with different mutations in GJB1 gene. Most mutations (96,8%) located in the coding region (in the 2 exone) of the GJB1 gene from 59 to 262 codon, advantage in second extracellular loop (26,7%), intracellular loop (16,7%) and first transmembrane domen (16,7%). One family have mutation in the noncoding region - IVS1+2T>C.
In 87,1% of our CMT 1X families we detected 21 previously reported missens point mutations: [I20G; N21S], V23A, W24R, M34K, Q80R, V84G, V91M, R142Q, R142Y, F193L, W44Q, W44C, C53Y, R164Q, V181M, R183H, E186K, M93V, A97P, R107W, E208K. 9,7% of our CMT 1X families had deletion, that produced frame shifts: 62delG, 606delC, 784-785delAT. Five patients from two families showed to our knowledge previously unreported base changes: 62delG and 606delC. In our family material we have not discover insertions, deletions and nonsense mutations.

P0720. Novel GJB1 mutation associated with mild phenotype CMT1X disease

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X-linked motor and sensory neuropathy has been found to be due to mutations in the GJB1 gene, mapping to Xq13.1 region, encodes for the gap junction protein - connexin 32. Of 230 mutations identified in CMT1X families, most are missense mutations. The phenotype of CMT1X has been divided into mild, moderate, and severe. Most missense mutations showed a mild clinical phenotype, whereas all nonsense mutations, the larger deletions and the insertion that produced frame shifts showed severe phenotypes. We report a novel mutation identified in Russian family. In this family with four

affected individuals, the 1 bp deletion - 62delG- in the codon 21 second nucleotide was found. The mutation predicts a shift in the reading frame and termination of translation after amino acid 83. The proband is a 25-year-old male who has a sings of a demyelinating sensorimotor neuropathy. His electrophysiological findings were typical for CMT1 type. Three another patients - his mother and two sisters -had only discrete walking difficulties and atrophy of the peroneal muscles. There electrophysiological findings indicating an axonal neuropathy.

P0721. Novel PKU mutation associated with mild phenilketonuria in patient from Voronezh region

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Phenilketonuria is an autosomal recessive disease caused by mutation in phenilalanin hydroxylase gene (PAH). Up to date it is known more than 400 mutations. We have studied more than 300 patients from different Russian region for mutations in 5, 7 and 12 exons of PAH gene. All find mutations except one are published in PAH mutations database. Here we describe the novel mutation in PAH gene in patient from Vorenezh region (Russian Federation) which were reviled by PCR-SSCP analysis with following sequence. We have reviled the nucleotide change G->A, which result in amino acid substitution G256D. This mutation was found in compound heterozygote with R408W mutation. Phenilketonuria usually is associated with a severe mental retardation especially without diet therapy. However this patient have not regular diet therapy in his early childhood but severe mental retardation were not determined.

P0722. Linkage study of a large CMT family from Udmurtia, Russian Federation

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Charcot-Marie-Tooth neuropathy (CMT) is a heterogeneous group of peripheral nervous system disorders with a broad spectrum of clinical severity and different pattern of inheritance. Autosomal dominant CMT is classified into two forms based on neuropathogloical criteria. CMT1 is characterized by demyelination and remielynation, while CMT2 exhibits axonal degeneration. To date at least 6 different genetic loci is known for CMT type 1 and 5 others for CMT type 2. Here we described large CMT family from Udmurtia, Russia with 11 affected persons in three generations. All of them have typical clinic neuropathy but type undetermined. The associated features of some patients are tremor of hands or signs of spno-cerebellar ataxia or both. We have curried out linkage analysis for some CMT loci to established disease type in this family. The D1S2663; D1S2667; D1S450; D1S228 markers were investigated for CMT2A; D8S136; NEFL-CA - for CMT2E; D7S2490; D7S2470; D7S675 - for CMT2F. All these loci as well as MPZ locus and gene and CMT1A locus were excluded as disease locus in this family. The investigation other CMT loci are in progress now but we suggesting the presence of additional CMT locus in this family.

P0723. Detection of mutations ΔF508, 2183AA>G, 574delA, D110H, R334W, 2043delG in Iranian Cystic Fibrosis by ARMS-PCR

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Cystic Fibrosis is an autosomal disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator(CFTR) gene,which may cause a common lethal disease. Since isolation of the gene more than 1000 mutations have been reported to the Cystic Fibrosis Analysis Consortium. The frequency of these mutations varies in different populations. Identification of mutations causing Cystic Fibrosis in the Iranian population is essential for assessment of the molecular basis of CF in Iran and development of strategies for prental diagnosis and genetic counseling. We have previously screened some of encoding regions of CFTR gene and identified six disease-causing mutations on 66 unreleated CF chromosomes from 33 Iranian Cystic Fibrosis families. In the present study, we disinged ARMS -PCR for detection these six mutations(ΔF508, 2183AA>G, 574delA, D110H, R334W, 2043delG). The frequency of these mutations is : 25.75%, 4.54%, 3.03%, 1.51%, 1.51%, and 1.51% respectively. This study showed that the developed ARMS-PCR method is a rapid, costbenefit and suitable assay for detection and determination of above mutations.

P0724. SNP-array mapping of a novel locus for autosomal recessive retinitis pigmentosa to chromosome 14q24

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Hereditary retinopathies represent the major cause of adult genetic blindness in the western world. Retinitis pigmentosa (RP) is a heterogeneous group of retinal dystrophies characterized by degeneration of the rod and cone photoreceptor cells. Therein, autosomal recessive RP (arRP) is the most frequent form. Today, the etiology and pathomechanism of the disease remain unknown in >= 85% of cases. Although causative mutations for arRP have been reported in about 30 different human genes, these explain only a minority (< 15 %) of arRP cases. We identified three consanguineous kindreds with a total of 13 patients affected by autosomal recessive nonsyndromic childhood-onset retinal dystrophy that were thought to belong to an extended Austrian pedigree. Whole genome scan by high-densitiy microarray analysis of SNPs mapped the disease locus to chromosome 14q23.3-q24.1, and a shared founder haplotype defined a critical interval of 1.53 cM. Using this approach, we rapidly detected a small region of homozygosity that might have been missed by conventional methods. Current mutation analysis of candidate genes from the critical interval will contribute to the revelation of the etiology and pathomechanism of this condition.

P0725. Investigation of mutations involved in macular corneal dystrophy

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Macular corneal dystrophy (MCD) is an autosomal recessive disorder leading to severe visual impairment. The carbohydrate sulfotransferase 6 (CHST6) gene, which encodes the corneal N-acetylglucosamine 6-O-sulfotransferase on 16q22 has been identified as a causative gene for MCD. We screened the *CHST6* gene for mutations in Iranian patients with MCD, in order to determine the range of pathogenic mutations. Genomic DNA was extract from peripheral blood leukocytes of 14 patients with MCD. The coding regions of the *CHST6* gene were amplified using three pairs of primers and amplified products were directly sequenced. Mutation analysis of the CHST6 coding region identified 10 different mutations in 14 Iranian patients with MCD. We found new heterozygote mutation in C1166G mutation in CHST6 gene by Sequencing. We suggest that sequencing technique is the best method for the population, which we do not know much about genetic background.

P0726. Molecular analysis of CFTR gene mutations in Iranian CF patients

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Cystic fibrosis (CF) is a common autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. More than 1000 mutations and 200 different polymorphisms have been reported in the CFTR

gene. It has been recognized as the most lethal genetic disease and frequently causing death in children.
The frequency of CF mutations is not known in Iranian population. To identify Iranian common CF mutations, we have studied 50 Iranian CF patients. All affected individuals were unrelated and diagnosed as having CF according to the world health organization criteria (WHO). The DNA of patients was isolated from blood cells by the salting out method. All patients were screened for 8 CFTR gene mutations which are common in European population by using allele specific PCR technique. The results showed that frequencies of these mutations in Iranian are different than European population. In ΔF508, the frequency was decrease to 12 percent as compared with 66 percent in the European, while, the frequency of N1303K increased to 5 % as compared with 1.5% in the European. However, we increased the number of the samples to 100 and our results will give more accurate results in the number and the frequency of mutations in Iranian population.

P0727. Germline deletion of the SH2D1A gene in a Russian family.

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X-linked limpoproliferative syndrome (XLP, Duncan's disease) is a rare inherited disorder characterised by a defective immune response to Epstein-Barr virus (EBV) infection. The gene responsible for XLP has been identified as the four-exon SH2D1A gene encoding a 128-amino-acid protein. This protein acts as a regulator of at least two signal transduction pathways initiated by the cell surface molecules SLAM and 2B4. The most frequent types of mutations are genomic and intragenic deletions.
We studied DNA of 1 affected male. Twelve-years-old patient died due to fatal EPV infection. Proband had fever, virus-associated lymphohistiocytosis, aplastic anemia, hepatosplenomegaly, hepatic failure. Diagnosis XLP was confirmed post-mortem using molecular genetic methods.
DNA was extracted from post-mortem histological sample conserved by paraffin and blood samples. PCR products of 1-4 exons of the SH2D1A and microcatellite marker in the 2 intron of the same gene were amplified using original primers. We identified complete deletion of SH2D1A gene in proband's DNA. Proband's mother and her sister had two copies of SH2D1A. Using microsatellite polymorphic markers we found that deletion has been located between Xq27 and Xq13. This is the first case of molecular diagnostics of the Duncan's disease in Russia.

P0728. Investigation on mutations of MYH7 gene in Iranian Hypertrophy cardiomyopathy (HCM) patients

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Hypertrophy cardiomyopathy (HCM) is a genetic disorder typically inherited in an autosomal dominant fashion with variable pen trance and variable expressivities. The disorder has been estimated to occur in 0.05-0.2% of the population.
Mortality rate for individuals with HCM is 4% per year. In this study, we vest igated on 50 blood samples . Our investigation is focused on mutation detection in bMHC gene in exons that have more common mutation. PCR is done by 13F and 15R primers and also by 19F and 21R primers to amplify respectively 847bp and 799bp fragments. These amplimers were sent for sequencing. Other exons which have more common mutation like exons 13, 14 15, 19, 20 and 21 and then would be sequenced. If there is high-risk mutation in the patient's family they would be referred to cardiologists for implanting ICD or drug therapy. In This study 52 HCM affected patients have been evaluated for mutations in exon 13-14-15-19-20-21 of MCH7gene with the aid of PCR and sequencing.
Result showed that 14 of 52 patients had mutation after study their sequencing chromatogram. Four of these 14 patients had mutation in exonic regions and the other patients in the intronic region. Founded exonic mutations in patients were GI0I95A in exon 13, A10419C in exon14, C13430T, C13978A in exon 19.
Further study in patient's families needs to reveal the role of these mutations.
It seems that mutation diagnosis in MYH7 gene can give us a good prognosis to prevent sudden death of affected ones.

P0729. Identification and sizing of GAA trinucleotide repeat expansion of Fridreich's Ataxia in 15 Iranian patients

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Friedreich's ataxia is a neurodegenerative disorder whose clinical diagnostic criteria for typical cases include a) early onset age, b) autosomal recessive inheritance c) progressive ataxia of limbs and gait and d)absence of lower limb tendon reflexes. It is the commonest genetic cause of ataxia and is associated with the expansion of a GAA repeat in intron 1 of the frataxin gene. Approximately 96% of patients are homozygous for this expansion mutation with 4% being compound heterozygotes for the repeat expansion and a point mutation.
We studied 15 Iranian patients (9 females and 6 males) from 7 unrelated families. DNA from each patient was extracted and frequency and length of $(GAA)_n$ repeat in the first intron of the gene FRDA was analyzed using a long-Range PCR test. Also we investigated impact of GAA size on neurological findings, age of onset and disease development.
Homozygous GAA expansion was found in 12 cases (80 %) all typical cases. In 3 cases (20 %), no expansion was observed, ruling out the diagnosis of Friedreich's ataxia. In cases with GAA expansions, ataxia, scoliosis and pes cavus, cardiac abnormalities and some neurological findings occurred more frequently than in our patients without GAA expansion. Molecular analysis was imperative for diagnosis of Friedreich's ataxia, not only for typical cases, but also for atypical ones. Diagnosis bases only on clinical findings is limited, however, it aids in better screening for suspected cases that should be tested.

P0730. Molecular studies on prevalence of LHON primary point mutations in Iranian patients

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Leber Hereditary Optic Neuropathy (LHON) is a maternally form of central visual loss that occurs sub acutely in young persons. The aim of this study was to define the prevalence of a panel of mitochondrial DNA (mtDNA) mutations associated with Leber Hereditary Optic Neuropathy (LHON) in Iranian LHON population. We studied four well-known LHON associated primary point mutations (at nucleotide positions 11778, 3460, 14484 and 14459) in 35 Iranian LHON patients. On the basis of our study 11 affected patients were positive for one of four primary LHON point mutation, 10 patients were males (91%) and one was female (9%). The G11778A was found in all the females (100%) of the patient's family and no one showed the LHON phenotypes. We were detected 72% for the G11778A mutation. 14% for the G3460A mutation, 7% for the G14459A point mutation and the 7% for the T14484C point mutation in our patients. Our results showed similarity of Iranian LHON families with Russian, European and North American. So, 10 new point mutations were detected in the rest of patients who had not any primary LHON point mutations in hot spot region for LHON disease (ND6 gene).

P0731. Detection in mtDNA deletion in infertile men.

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Mitochondrion is an intracellular of organelle that present between 10 to1000 in every cell. Mitochondrion contains a double - strand

circular DNA with 16,569 nucleotide pairs. Every mitochondrion has 2 - 10 mtDNA copies. The important role of mitochondria is energy production. This energy was produced by oxidative phosphorylation reaction. There are 75 - 100 numbers of mitochondria in midpiece region of sperm. The role of these mitochondria is ATP producing for sperm motility and mobility, which is important for fertilization. MtDNA mutations cause difficult in energy producing of process and results disorder in motility of sperm. Deletion in mtDNA of 100 infertile men were investigated by standard and multiple PCR. We were found 3 patients with mitochondrial deletions. The deletion sizes were 7Kb Break point of deletions was according to sequencing. Southern blot analysis needs to detect the percentage of deleted mtDNA in those patients.

P0732. Isolation of three novel disease genes indicate heterogeneity but clustering of XLMR genes in the Xp21.1-11.21 region

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Based on a systematic screening approach within the scope of the DHGP2 project, we identified three novel disease genes causing both syndromic and nonsyndromic forms of X-linked mental retardation (XLMR). MRX9, a large Belgian family with mild to severe non-syndromic mental retardation, was found to have a mutation in a human homologue of the bacterial 23S-rRNA methyltransferase Ftsj, FTSJ1. The mutation is located in the conserved acceptor splice site of intron 3 (IVS3-2A>G) and results in the skipping of exon 4 and the introduction of a stop codon in exon 5. In E. coli Ftsj could be associated with ribosomal stability. A family with mental retardation and epilepsy (XMRE) was found to have a mutation in a putative exonic splice enhancer site for the ATP6AP2 gene (see Ramser et al.). Additionally the original Renpenning syndrome family, characterized by short stature, microcephaly and XLMR, was found to have a mutation in exon 5 of PQBP1 gene (see Schwartz et al.). The idea that XLMR exhibits profound heterogeneity with a clustering of disease genes in Xp11.4-11.21 is supported by the lack of mutations in these three genes in many other linked families and in two other recently identified XLMR genes: ZNF41 and ZNF81. Our data also indicate that except for PQBP1 or ARX, most XLMR genes are rarely mutated in linked families. Nonetheless, the identification of FTSJ1, ATP6AP2 and PQBP1 expand the pathways associated with XLMR and their functional characterization should elucidate novel mechanisms in cognitive development.

P0733. Molecular analysis of the (CAG)n repeat causing Huntington‘s disease in 34 Iranian families.

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We have analyzed the distribution of CAG and adjacent polymorphic CCG repeats in 71 Iranian individuals (34 patients and 37 unaffected family members) belonging to 31 unrelated families thought to segregate *HD*. We found one expanded CAG allele in 22 individuals (65%) belonging to 21 unrelated families. In these *HD* patients, expanded alleles varied from 40 to 83 CAG units and normal alleles varied from 13 to 36 CAGs. A significant negative correlation between age at onset of symptoms and size of the expanded CAG allele was found (r= - 0.51; p=0. 1); however, the size of the expanded CAG repeat could explain only about 26% of the variability in age at onset (r^2=0.26). In addition, we genotyped 25 unrelated control individuals (total of 50 alleles) and found normal CAG repeats varying from 10 to 34 units. In conclusion, our results showed that not all patients with the „*HD*“ phenotype carried the expansion at the *IT15* gene. Therefore, molecular confirmation of the clinical diagnosis in *HD* should be sought in all suspected patients, making it possible for adequate genetic counseling. This Study is the first report of molecular diagnosis of Huntington disease among Iranian population and maybe in Middle East and with regard to high frequency of consanguinity marriage in this region, we thought the frequency of this disease will be more than expected amount for this geographical region.

P0734. CFTR mutations in Ecuador

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Cystic fibrosis is a common hereditary recessive disease that affects 2500 of new born in Caucasoid population. In Ecuador there is only one report for DF508 mutation in 20 patients with cystic fibrosis. In this group it was described an allelic frequency of this mutation of 26 % using Innolipa CF9.
The present study analyzed 60 patients with cystic fibrosis diagnosed at the two principal cities in Ecuador, Quito and Guayaquil. There are many techniques for analyzing CFTR gene mutations, some of them designed for a specific area. This work used the ASO technique (Innolipa).
This is the first screening to detect CFTR gene mutation in our country, we had two objectives: to determine percentage of already described mutations and to identify new mutations in order to design the best test to permit in the future a rapid screening in our population.
With this method, we detected 59,8% of mutant alleles (DF508 35%, G85E 15%, G551D 3,3%, G542X 3,3%, N1303K 1,6%, R334W 1,6%). The unknown mutations are being studied using DGGE technique and until now the most polymorphic zone is exon 6, but the sequences are not yet ready.

Mutations	Percentage
DF508	35%
G85E	15%
G551D	3,3%
G542X	3,3%
N1303K	1,6%
R334W	1,6%
Percentage of mutant alleles	59,8%

P0735. Osteopontin gene mutation screening in patients with urolithiasis

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Osteopontin (Opn) is multifunctional molecule which is involved in biomineralizatin, leucocyte function, leucocyte recruitment, inducible nitric oxide syntheses (iNOS) in both macrophage and primary renal tubular epithelial cells, cell survival, wound repairement, and tumor invasion and metastatic spread. Cells bind Opn through integrin receptors. Integrin binding may be RGD (Arg-Gly-Asp) dependent or independent via SVVGLA (Ser-Val-Val-Tyr-Gly-Leu-Arg) motif. Familial and sporadic urolithiasis patients including control group were investigated by SSCP analysis for Opn gene locus. Opn gene is located on chromosome 4q13 and consists of 7 exons. The nucleotide sequence between 3455-9445 bp is the coding region. The mutation screening which is the part of ongoing work, showed deviation in mobilities between 1575-1830 bp (OstP6), 2125-2290 bp (OstP), and 9015-9371 bp (OstEx7a). Among the five mutations two in OstP6, one in OstP, and two in OstEx7a region, only one in OstP region can be suspected for urolithiasis, because others were also either observed in or found control group. OstP region is the binding region of E4TF1 (nuclear respiratory factor-2 subunit beta 1), E2A (basic helix-loop-helix transcription factor), SP-1 (transcription factor), AP-1 (transcription factor binding DNA-site), Ets1 (E26 avian leukemia oncogene 1,5’domain), and TATA box.

P0736. Unexpected high frequency of somatic mosaicism in patients with NF1 microdeletions caused by non-allelic recombination between low copy repeats (LCRs)

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The importance of low copy repeats (LCRs) as mediators of erroneous meiotic recombination generating constitutional chromosome rearrangements is well established. However, the significance and frequency of somatic recombination between LCRs is not clear. We observed an unexpected high frequency of mosaicism in patients with NF1 microdeletions caused by somatic recombination between LCRs in the NF1 gene region. In 7 of 19 patients investigated (37%), mosaicism of normal cells and those with NF1 microdeletions was detected. Our studies also show that NF1 microdeletions are divided in two major subgroups with respect to the breakpoints within the LCRs: In 11 of 19 deletions (58%), the breakpoints map to the WI-12393-derived LCR subregion. Deletions of this type span 1.4 Mb and include 14 genes inclusive of the NF1 gene. The second type of NF1 microdeletion, observed in 8 of the 19 cases (42%), has breakpoints in the JJAZ1 sequences within the LCRs. Deletions of this type (class II) encompass 1.2 Mb and span 13 genes. Using somatic cell hybrids that contain only one recombinant LCR, we mapped the breaks within the JJAZ1 sequences. Astonishingly, in the majority (85%) of patients with mosaicism, the deletions belong to class II. Therefore, the JJAZ1 gene is a preferential target for strand exchange in somatic recombinations leading to deletions of 17q11.2. The high rate of mosaicism in NF1 microdeletions has to be considered in the context of genotype/phenotype correlations and also implies an important role of the NF1 LCRs in somatic rearrangements.

P0737. A novel *GDAP1* mutation 439delA is associated with autosomal recessive Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is the most common form of inherited motor and sensory neuropathy. CMT has been classified into two main types, the demyelinating and the axonal, based on neurophysiological and neuropathological criteria. It may be inherited in an autosomal dominant, autosomal recessive (AR) or an X-linked form. Seven loci/genes have thus far been identified for the demyelinating AR-CMT disease. These are: CMT4A mapped on chromosome 8q13-q21.1, caused by mutations in the *GDAP1* gene; CMT4B1 on 11q22, caused by *MTMR2* mutations; CMT4B2 on 11p15, caused by *SBF2* gene mutations; CMT4C on 5q32, caused by *KIAA1985* mutations; CMT4D on 8q24.3, caused by mutations in the *NDRG1* gene; CMT4E on 10q21.1-q22.1, associated with *EGR2* mutations; and CMT4F on 19q13.1-q13.2, associated with *PRX* mutations.

We describe a consanguineous AR-CMT family of Iranian descent with two affected young girls. The proband with disease onset at $2^{6/12}$ showed evidence of a demyelinating neuropathy by electrophysiological and neuropathological studies, whereas her younger sister with disease onset at 2 years old showed electrophysiological evidence of an axonal neuropathy. We linked the family to the CMT4A locus and identified a novel *GDAP1* mutation (439delA) that is associated with the disease. The above mutation results in an alteration in GDAP1 from amino acid 147 and it is predicted that a premature termination occurs subsequent to amino acid 151.

This study was financially supported by the Muscular Dystrophy Association (USA).

P0738. Molecular analysis of the L-ferritin 5'UTR: identification of a novel mutation in two unrelated hereditary hyperferritinaemia cataract syndrome cases

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Hereditary Hyperferritinaemia cataract syndrome (OMIM #600866) is an autosomal dominant disorder characterized by persistently high levels of serum ferritin not related to iron overload and by bilateral nuclear cataract that arises at different ages. It is caused by mutations in the iron- responsive element (IRE) located in the 5-prime non coding region of the ferritin light-chain gene. Several different mutations, able to interfere with the affinity binding, at mRNA level, between IRE and iron regulatory proteins (IRPs), with consequent constitutive up-regulation of ferritin light-chain, have been described to date.

Genotype-phenotype correlation have been established, but it only partially explains the marked phenotypic variability observed in the disorder.

We have identified the novel 29C-G mutation in the IRE of the L-ferritin gene in two unrelated Italian individuals. The first patient is a 45 year old man with serum ferritin levels of 550- 850 µg/l and bilateral "pulverulent" asyntomatic cataract. The second patient is a boy, aged 7, referred for persistent hyperferritinaemia (520-600 µg/l), in whom ophthalmological evaluations have not revealed to date the presence of lens opacities. Both the cases are apparently sporadic. The 29C-G substitution involves an highly conserved, paired cytosine located in one of the lower stems of the IRE structure, below the region where recurrent mutations are present. It seems to act as a "mild" mutation being associated with moderate increase of serum ferritin and if present, with subclinical not very precocious cataract.

P0739. Identification DelA3314 in mitochondrial ND1 gene in diabetic type 2 patients

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MtDNA has 16.5 kb that carries 37 genes, including 2 RNAs, 22 tRNAs, and 13 polypeptide subunits of enzymes interfering with the oxidative phosphorylation. There is a variant of mtDNA mutations which is A3243G tRNA(leu).

While high level (90%) of the A3243G mutation associated with MELAS but low level (50%) of this mutation related to diabetes mellitus type 2 with or without Deafness. 5kb mtDNA deletion has been also reported in the patient's with diabetes mellitus type 2. We assessed the frequency of the A3243G and 5kb mtDNA deletion in Iranian diabetes mellitus type 2. DNA was extracted form blood of 140 diabetic type 2 patients. Insulin rate of the patients were also tested. PCR-RFLP and SSCP methods were used to detect the A3243G or other mutations in the mitochondrial tRNA(leu) gene. Standard and multiplex PCR were used to detect to 5kb deletion in patient's mtDNA. We could not identify any deletion or A3243G point mutation in our cases. SSCP results showed new pattern of PCR product in 7 patients. Sequencing was done by 3700 ABI capillary system. An "A" nucleotide deletion in 3314 position was detected in mitochondrial ND I gene. So far' this deletion has not been reported.

P0740. Spectrum of mutations and single-nucleotide polymorphisms in Long-QT Syndrome genes KCNQ1, KCNH2 and KCNE1.

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The Long QT syndrome (**LQTS**) is an inherited cardiac disorder in which ventricular tachyarrhytmias 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: **LQT1** (KCNQ1/11p15.5), **LQT2** (KCNH2/7q35-36), **LQT3** (SCN5A/3p21-24), **LQT4** (Ankyrin-B/4q25-27), **LQT5** (KCNE1/21q22.1-22.2) and **LQT6** (KCNE2/21q22.1). Mutations in potassium-channel genes KCNQ1 and KCNH2 are the most common cases of the LQTS.

We used single strand conformational polymorphism (**SSCP**) analysis to screen all exons of **KCNQ1, KCNH2** and **KCNE1** gene in group

of unrelated Czech LQT patients. SSCP analyses were followed by sequency analyses of abberant conformers.
DNA sequence analyses determined one frameshift mutation (P631fsX650) and three missense mutations (G325R, G350A and T309I) of KCNQ1 gene. Mutations T309I and G350A were novel. Mutation analyses of KCNH2 gene revealed three missense mutations: R534C, A448T and A228V. Mutations A448T and A228V have not been published yet. We also identified eight different single-nucleotide polymorphisms (SNPs) in KCNQ1 and KCNH2 and two variants of introns in KCNH2 gene. The screening for mutations in the KCNE1 gene revealed rare amino acid variants G38S and D85N.
The potential role of rare polymorfhisms and **rare allelic variants** in the ion channels remains to be clarified with respect to drug interactions and susceptibility to arrhytmia and sudden death in common population.
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P0741. Segregation of a splice acceptor mutation IVS3-9 in the COL1A1 gene in a three generation OI type I family

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Osteogenesis imperfecta (OI) is a heritable disorder of bone formation resulting in brittle bones. More than 90% of patients with OI are thought to have mutations in the COL1A1 or COL1A2 genes coding for Type I procollagen. About two thirds of the mutations result in substitutions of glycin in the triple-helix forming domain. The remaining third is composed of frame shift and splice site mutations. We report the case of a patient with the mild form of OI type I lacking any classical mutation but exhibiting two potential splice site mutations identified by complete sequencing of both genes: IVS12+5A>T in the COL1A2 gene and IVS3-9A>G in COL1A1. Both changes do not alter highly conserved residues in intronic splice sequences. RT-PCR analysis of transcripts of a fibroblast culture of the patient revealed an aberrant COL1A1 splice product with an insertion of the last 8 bp of intron 3 resulting in a translational frame shift. This is due to the creation of an alternative 3' splice acceptor site in intron 3 by the single-base change at position -9. In addition, the analysis of several affected and unaffected family members showed segregation of the COL1A1 IVS3-9 change with the disease in three generations and supported the hypothesis that IVS3-9A>G represents the disease causing COL1A1 mutation.

P0742. A novel Notch3 gene mutation not involving a cysteine residue in an Italian family with CADASIL

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) is a hereditary cerebrovascular disease leading to accumulating neurologic deficits and dementia. CADASIL usually presents with early onset strokes in 43% of patients. Additional manifestations include progressive subcortical dementia in 6%, psychiatric disturbances in 9%, and migraine in 40%. Epilepsy has been reported in 2-10% of patients, most often following strokes.
CADASIL has been linked to nucleotide substitutions and deletions in the Notch3 gene. CADASIL is caused by single missense mutations, small in-frame deletions, or splice site mutations in the Notch3 gene encoding a transmembrane receptor. All previously reported mutations resulted in an odd number of cysteine residues within one of the 34 epidermal growth factor (EGF)-like repeats in the extracellular amino-terminal region of the Notch3 receptor. We report the first evidence of a small deletion, which did not directly involve a cysteine residue, located within the extracellular domain of the Notch3 gene causing CADASIL in a family from southern Italy. This deletion is predicted to result in a loss of four amino acids (88-91) segregated with the neurologic and clinical phenotype suggesting that it is the causative mutation in the our study family.
The current findings demonstrate that the mutations not involving the cysteine residue in the Notch3 gene can cause CADASIL. This finding provides new clues for the investigation of the mechanism leading from Notch3 mutations to the CADASIL phenotype.

P0743. Genetic characterization of NBS-like patients defective in double strand breaks repair mechanism

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We studied lymphoblastoid cell lines from 6 Njiemegen breakage syndrome - like Italian patients with growth delay, immunodeficiency and microcephaly. Defect in the repair of DNA double strand breaks (DSBs) can lead to profound immunodeficiencies.
Mutations in the *Nbs1* gene lead to the Nijmegen breakage syndrome (NBS), an autosomic recessive disorder characterised by a bird-like face, immunodeficiency, high frequency of malignancies, chromosome instability, sensitivity to ionising radiation and abnormal cell-cycle checkpoints.
We studied cellular and molecular characteristics of our LCLs. PCR amplification of exon 6 showed the presence of both copies of the *NBS1* gene in all the investigated individuals. Furthermore, normal levels of proteins involved in the repair of DSBs (M/N/R complex, Lig IV, XRCC4) were detected. Co-immunoprecipitation assay confirmed the capability to form a proficient M/N/R complex. Radiosensitivity of some of the LCLs analysed was shown by the induction of chromosome aberrations in a G2-phase assay.
After cells irradiation, p53 proficiency in DNA-damage response pathway was ascertained by immunoblotting either with a p53 antibody or a specific antibody for the phosphorylated form p53-p-Ser15.
Experiments are in progress to measure DSBs rejoining by means of pulsed-field gel electrophoresis and more than hundred genes involved in the response to DNA- DSBs will be analysed by means of gene-array assay. Dissection of the clinical and cellular phenotype of these as well as future NBS-like patients will help to identify a subset of individuals with the NBS clinical phenotype.
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P0744. Molecular study of ABCA4 gene on Spanish patients with retinal dystrophies: preliminary results.

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Mutations in ABCA4 gene are associated to five distinct phenotypes: Stargardt disease, fundus flavimaculatus, retinitis pigmentosa, cone-rod dystrophy and age related macular degeneration. Direct analysis of ABCA4 gene has important parameters to consider:
- extraordinary allelic heterogenity (with low frequency for each mutation)
- variable rate of mutation (25-80%)
- the large size of the gene (50 exons, 6800 pb open-reading-frame)
68 Spanish patients with Retinal Dystrophies were screened for mutations in ABCA4 using genechip (Asperbio Ltd), followed by haplotype analyses in some cases.
The gene chip could establish 54 disease-associated alleles out of 136 chromosomes (40%). We found 5 complex alleles. The most frequent reported allele, 5882G>A was observed in similar proportion as in other populations (4, 41%), however the most frequent mutation in our study was 3386G>T with 8,82% of the allele disease associated.
We detected both disease-associated ABCA4 alleles in 26%, one allele in 30, 8% and no mutation was found in 42% of the families. The ABCA4 microarray detects only known variants and is currently expected to detect 56% of the disease-associated alleles in populations of European origin.
Therefore, for molecular diagnosis purposes, it is necessary to correct the mutation rate detection adjusted to Spanish frequencies for any of the associated phenotypes. The combination of this method with other screening mutation methods or linkage family studies, in addition to the inclusion of more mutations, will considerably increase the sensitivity to detect disease associated alleles.

P0745. Efficient detection of heterozygous intragenic ZFHX1B deletions by semi-quantitative fluorescent multiplex PCR improves molecular diagnosis of Mowat-Wilson syndrome

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Heterozygous mutations or deletions involving ZFHX1B (SIP1) have recently been found to cause one form of syndromic Hirschsprung disease (HSCR), associated with microcephaly, mental retardation, and distinctive facial features, called Mowat-Wilson syndrome. Patients with the characteristic facial phenotype and severe mental retardation, but without HSCR, have also been shown to have mutations in this gene. We have so far identified 40 truncating mutations in more than one hundred patients studied. There are patients presenting with the characteristic phenotype, who are likely to have Mowat-Wilson syndrome, but who do not display any ZFHX1B intragenic point mutations or another major chromosomal abnormalities at the 2q22-23 locus. Indeed, heterozygous intragenic deletions escape the routine PCR-sequence analysis procedure currently used by most laboratories, and this prompted us to develop a mutation analysis procedure suited to circumvent this difficulty. We developed a semi-quantitative fluorescent multiplex PCR assay (QMF-PCR) in which a unique multiplex fluorescent PCR amplification of all ten ZFHX1B exons is carried out under conditions that allow rapid and reliable quantitative comparison of the fluorescence of each amplicon in test samples and in controls. Using this procedure, we could show that several patients, in which no mutations were previously found, bear in fact deletions or other small rearrangements within ZFHX1B. More than 50% of ZFHX1B defects identified so far are insertion/deletion of 1-4 bp. As this efficient method allows detection of single nucleotide deletion/insertion, it has now replaced the superseded PCR-sequence assay in the strategy used in our laboratory to study this syndrome.

P0746. Application of a high-resolution subtelomere array in research and diagnosis.

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Subtelomeric rearrangements are a significant cause of idiopathic mental retardation, but are often difficult to recognize with standard cytogenetic banding techniques. FISH analysis of all subtelomeric regions is time-consuming and provides no information about the extent of the aberration. As arrayCGH allows for sensitive analysis of copy number changes, we constructed a high-resolution array for the detection of unbalanced subtelomeric rearrangements. The latest version of this array covers the first 5Mb of each subtelomere with 8-12 BAC clones, while the first 1 Mb approximates tiling path resolution. A total of 432 autosomal BACs, 40 chromosome X-specific, and 3 chromosome Y-specific BACs, all linked to the human genome sequence, are present on the array. The obtained data are analysed with specifically designed software. We present the results of a validation study on 40 samples of which cytogenetic and FISH data were available. As an example illustrating the power of this array, we present the case of a 20 year-old mentally retarded female patient with short stature and dysmorphic features. Cytogenetic analysis of the patient's blood lymphocytes revealed 46,XX,add(3)(p26). FISH, however, revealed loss of the 3p subtelomere and complete staining with wcp3. ArrayCGH indeed confirmed the heterozygous deletion of the terminal subtelomeric region of chromosome 3p, which appeared to be 4 Mb. In addition, the adjacent 1.3-Mb segment appeared to be present in seven copies. Further analyses with a high density chromosome 3-specific array indicated a duplication of the subsequent 6-Mb segment. The gain of material probably explains the cytogenetic observations.

P0747. Mutational Screening of the Notch3 Gene in Patients from Southern Italy Affected by CADASIL: Identification of Four Novel Pathogenic Mutations

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Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL; MIM#125310) is a late-onset syndrome characterized by subcortical ischemic strokes, attacks of migraine with aura, and vascular dementia. Subcortical dementia, in all cases associated with pseudobulbar palsy, is the second commonest clinical manifestation of CADASIL. All individuals, both symptomatic and asymptomatic, have prominent signal abnormalities on brain Magnetic Resonance Imaging (MRI).
CADASIL is caused by mutations in the Notch3 gene encoding a transmembrane receptor. The contribution of Notch3 mutations to the italian population incidence of CADASIL was unknown. In the present study, twenty-five patients from 10 families of South Italy have been clinically examined and submitted to neuroimaging studies. Molecular investigation has been performed on 22 of 33 exons of the Notch3 gene encoding for the extracellular domain, containing the 34 Epidermal Growth Factor (EGF)-like repeats, by DHPLC and following direct sequence.
Ten pathogenic mutations have been detected: two in the exon 3 (2x R90C), two in the exon 6 (2x R322C), four in the exon 8 (C428T, C428R, C440S, R449C), one in the exon 10 (G528C), and one in the exon 22 (R1231C). Three mutations in the exon 8 and one in the exon 10 are new. All these missense mutations involve gain or loss of a cysteine residue. Furthermore, we detect 14 different polymorphisms, 7 out of 13 were nonsense substitutions, 5 were amino acid changes, 2 intronic variations.

P0748. FTSJ1: a novel player in X-linked mental retardation

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Non-syndromic X-linked mental retardation (MRX) is a highly heterogeneous condition, and most of the relevant genes are still undetected. We have shown that 1/3 of the missing mutations cluster on the proximal short arm of the X-chromosome (Ropers et al., 2003), and mutation screening of candidate genes from this interval revealed that mutations in the polyglutamine binding protein 1 (PQBP1) gene can result in syndromic and non-syndromic XLMR (Kalscheuer et al., 2003). Here we report that FTSJ1, a member of the FtsJ/RrmJ family of RNA methyltransferases, is another novel gene for MRX. We identified splice site and truncating mutations in three families. Northern blot analysis of RNAs from affected males revealed a significantly lowered expression of mutant FTSJ1 transcripts. One of these mutations results in skipping of exon 9, which encodes the C-terminal part of the methyltransferase fold domain. Transfection experiments with mutant FTSJ1 showed that deletion of the relevant amino acid stretch leads to a markedly change of intracellular distribution of the FTSJ1 protein.
We are currently testing additional XLMR families for mutations in FTSJ1 and are trying to gain more insight into FTSJ1 function. In yeast, three homologous FTSJ1 proteins are known. The deletion strains for these genes are presently being used for complementation studies. Furthermore, by performing RNA in situ hybridization experiments, we investigate developmental expression patterns of the FTSJ1 mouse orthologue and of fly paralogues.

P0749. Aprataxin Mutations in Italian Patients with Ataxia and Oculomotor Apraxia (AOA)

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Between the patients referred to our center for Ataxia Telangiectasia (AT), about 20% show a 'variant' phenotype, i.e. absence of telangiectasia, reduced radiohypersensitivity, normal alphafetoprotein levels, late onset and slow progression of the disease. In most of them, no ATM mutations and normal levels of ATM protein were found.
Ataxia with oculomotor apraxia (AOA) shares with AT many of the neurological features but no telangiectasias neither radiohypersensitivity; alphafetoprotein is elevated in AOA type 2 but not in AOA type 1 patients, characterized by hypoalbuminemia followed by hypercholesterolemia. Patients with AOA1 have mutations in the aprataxin (APTX) gene, whilst patients with AOA2 are mutated in the senataxin (SETX) gene.
We studied by DHPLC the exons 5 and 6 of APTX gene in 20 suspected AT patients but negative for ATM mutations; they showed oculomotor apraxia and normal alphafetoprotein levels. In 4 of them we found APTX mutations in homozygosity; three presented the described 837G>A base change in exon 6, the fourth a new mutation in exon 5. In another patient a base change not resulting in truncation was found; we are now examining 100 controls to exclude a polymorphism. Noteworthing, all our AOA1 patients presented with telangiectasias, suggesting the possibility that the disease could be more clinically heterogeneous than believed.

P0750. Analysis of Connexin 26 (GJB2) and Other Important Gene Mutations in Turkish Families with Consanguineous Marriages: Preliminary Findings

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It has been shown that the Connexin 26 gene plays the major role in the formation of Autosomal Recessive Non-Syndromic Hearing Loss (ARNSHL). The connexin 26 gene has more than 90 mutations causing ARNSHL. 35delG mutation is responsible from the 70% of all mutations. In our study we have selected 14 families having consanguineous marriage and each family having at least more than one deaf child and non of the parents had hearing impairment. We performed DNA sequencing for the coding region of the connexin 26 gene to all of the 28 deaf patients. Within these 28 deaf children, 4 were homozygous and 1 was heterozygous for 35delG mutation, 2 were homozygous for delE120 mutation, 1 was heterozygous for M163V mutation and 4 were heterozgous for V153I polymorphism. In addition we sequenced the normal hearing family members of the patients whom we identified as having mutations As a result, 5 were heterozygous for 35delG mutation, 2 were heterozygous for delE120, 1 was heterozygous for M163V and 3 were heterozgous for V153I polymorphism. From those which had no connexin 26 mutation we have selected 1 deaf patient from such families and sequenced the 5'UTR region of the connexin 26 gene and also analysed for the large deletion in the Conexxin 30 gene but we were not able to find any mutation. The GJB2 is the most frequently mutated gene in ARNSHL and molecular diagnosis of this gene will give the patient the opportunity to receive a better counselling

P0751. Characterization of the human *LMX1B* promotor and search for kidney specific regulatory elements

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The Nail-Patella syndrome (NPS) is a rare autosomal-dominant disorder characterized by typical skeletal abnormalities and, in some cases (~50%), nephropathy. Nephropathy is the most serious aspect of NPS since it develops toward end-stage renal disease in approximately 15% of cases. NPS is caused by heterozygous loss of function mutations in the *LMX1B* gene, encoding a member of the LIM homeodomain protein family. Interestingly, only 50-60% of NPS patients develop nephropathy in spite of identical or similar *LMX1B*-mutations. It is suggested that yet unknown genetic modifiers in the mixed genetic background may influence the renal phenotype of NPS patients.
In an attempt to identify modifying factors of LMX1B action, we started to characterize the promotor region of the human *LMX1B* gene and to search for kidney specific regulatory regions. Extensive computer based analysis and interspecies sequence comparison using the available genomic sequences of human, mouse, chicken and fish revealed several conserved regions upstream of exon 1 and within intron 2. From EST-data and RT-PCRs we have evidence that some of these upstream regions most probably correspond to a novel gene adjacent to *LMX1B* in antisense direction (*ALH*). To further characterize possible regulatory regions, we mapped the transcription start point of *LMX1B* by RACE and primer extension analysis. *In silico* analysis of the predicted promotor region revealed several evolutionary conserved putative transcription factor binding sites which are quite common in other known podocyte specific genes, e.g. Nephrin. Usage and further functional characterization of these binding sites is in progress.

P0752. Identification of Genetic Loci Associated with Familial Frontotemporal Dementia

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Frontotemporal dementia (FTD) is a frequent cause of dementia, accounting for 8-10% of pre-senile cases. In different clinical series, 20 to 50% of cases have a family history of FTD (FFTD). Mutations in the MAPT gene are responsible for between 9 to 14% of all FFTD cases. The genetic cause of the remaining FFTD pedigrees is not yet known. Linkage analyses of large pedigrees indicated mapping of FTD to four loci. The objective of this project is to identify genetic loci associated with FFTD. In order to achieve this aim, we performed linkage analysis in large pedigrees, which were negative for MAPT mutations. Four large pedigrees and two small ones have been enrolled in the study. A positive LOD score for markers D17S791 and D17S951 (2.903, 2.879) could be obtained for the largest pedigree (Brescia-F071), in the region of the MAPT gene. No deletion or duplication of exon 10 of MAPT gene was present in any of the affected members. In conclusion, we identified a pedigree, Brescia-F071 that, similarly to at least other five families described in literature, display a linkage to chromosome 17q21, in the absence of MAPT gene mutations.

P0753. The Role of GHR mutations in Idiopatic Short Stature

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The term idiopathic short stature (ISS) describes children with a height of more than two standard deviations below the mean, normal or slow height velocity, normal birth weight, absence of specific endocrine abnormalities, and no evidence of chronic physical or psychological illness.
In some ISS patients the presence of partial growth hormone (GH) insensitivity was inferred from the low levels of GH binding protein. As it has been hypothesized that some of the children with diagnosis of ISS may bear heterozygous mutations of the GH receptor (GHR), we analyzed the 9 coding exons of GHR gene in 37 patients with ISS. We identified a novel heterozygous transition T>C of the codon 2 of aminoacid 162 (144) resulting in the missense mutation V162A (V144A). This mutation was not found in 100 control chromosomes. In one patient we identified in exon 8 a novel transition C>T of aminoacid 112 determining the synonimous change C112C (C94C). The previously described single nucleotide polymorphism A>G of codon 3 of aminoacid 186 (168) in exon 9 resulting in the synonymous change G186G was demonstrated in 22 ISS patients (12 homozygous and 10 heterozygous) and in 23 controls (16 homozygous and 7 heterozygous). The relative allele frequency was

similar in patients and in controls.
Our data suggest that some mutations in the heterozygous state may have some effect on stature, but since other factors influence the biologic effect of a mutation on linear growth more investigation are needed to clarify the role of GHR mutations in ISS.

P0754. Structure-function analysis of bestrophin

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Best vitelliform macular dystrophy (BMD) is an autosomal dominant disease with juvenile onset that causes loss of visual acuity.
The vitelliform macular dystrophy type 2 (VMD2) gene has been associated with BMD and encodes a 585 amino acid transmembrane protein termed bestrophin. It is thought to be involved in ion transport across the basolateral membrane of the retinal pigment epithelium. Mapping of transmembrane topography by N-glycosylation and insertion of protease cleavage sites suggests that bestrophin contains four transmembrane domains (TMDs) with one additional hydrophobic segment immersed into the bilayer and the N- and the long C-terminal portions facing the cytosol. The vast majority of the known disease-associated alterations are missense mutations which are non-randomly distributed along the protein clustering near the predicted TMDs. The mechanism by which the missense mutations cause disease is not clear.
In order to understand the molecular pathology of bestrophin a mammalian heterologous expression system was established. Twenty-eight missense mutations together representing the range of BMD-associated alterations were generated by site-directed mutagenesis and were transiently expressed in EBNA 293 cells. In addition, the five cysteine residues of bestrophin were systematically mutated to serine moieties. Immunocytochemical and biochemical analyses of the mutated proteins are aimed to clarify the influence of amino acid alterations on the subcellular localization and membrane topology. In addition, a series of co-immunoprecipitation experiments with wild type and mutated bestrophin defines the precise region necessary for oligomerization. This study is expected to contribute to a better understanding of the mechanisms underlying BMD pathogenesis.

P0755. Deletion of *LETM1* - a cause for mitochondrial dysfunction in Wolf-Hirschhorn syndrome patients?

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The leucine zipper, EF-hand containing transmembrane protein 1 (LETM1) has recently been cloned in an attempt to identify genes deleted in Wolf-Hirschhorn syndrome (WHS), a microdeletion syndrome characterized by typical facial dysmorphic features, severe growth and mental retardation, hypotonia and seizures. *LETM1* is deleted in almost all patients with the full phenotype and has recently been suggested as a candidate gene for seizures in WHS patients. *LETM1* encodes a protein with a transmembrane domain, two possible EF-hand motifs, a leucine zipper, several coiled-coil regions and a putative SAP-domain. Using LETM1-EGFP fusion constructs and an anti Letm1 polyclonal antibody we could recently demonstrate that LETM1 is located in the mitochondria.
Here, we present further data on putative protein domains of LETM1 and a detailed sequence comparison of LETM1. LETM1 is evolutionary conserved throughout the eukaryotic kingdom with a strong conservation of specific protein domains. LETM1 exhibits homology to the mitochondrial yeast protein *MDM38.* We observed an interesting similarity between the morphology of mitochondria from LETM1 overexpressing cells and from the yeast *MDM38*- ko-strains reported by Dimmer et al. (2002). MDM38 is proposed to be involved in organelle morphology and our results suggest that in higher eukaryotes LETM1 may at least in part exert a similar function. This function might be linked to calcium homeostasis in higher eukaryotes since, in contrast to yeast, LETM1 contains EF-hand motifs. We hypothesize that seizures as well as some other neuromuscular features of WHS patients may be caused by mitochondrial dysfunction resulting from a *LETM1* deletion.

P0756. A novel missense mutation in Human TTF-2 (FKHL15) gene responsible for Congenital Hypothyroidism

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Congenital Hypothyroidism (CH) is a relatively common congenital disorder occurring in about 1 of 3000-4000 live births. Thyroid dysgenesis (TD) is the most frequent cause of CH (85% of cases) with the thyroid either absent, greatly reduced in size or ectopic. The pathogenesis of TD is as yet unknown, and the disease is usually regarded as sporadic with a female predominance. The data from knockout mice have demonstrated the roles of several genes in thyroid organogenesis [thyroid transcription factors (TTF-1 and TTF-2), Pax 8, and TSH receptor], and their impairment has been occasionally reported in CH cases. TTF-2 is a member of the forkhead/winged helix-domain protein family and regulates the transcription of target genes such as thyroglobulin and thyroid peroxidase.
Two missense mutations (A65V and S57N) of human TTF-2 gene have been reported in four patients with CH. Here, we describe a CH case homozygous for a novel missense mutation (R102C) in the forkhead domain of TTF-2 gene. She was born to consanguineous parents, with bilateral choanal atresia, cleft palate and spiky hair. Her heterozygous parents were unaffected and 50 control chromosomes tested negative for the same mutation. The substitution at a highly conserved residue in the forkhead domain suggests that the mutant TTF-2 protein in the patient results in impaired DNA binding and complete or partial loss of transcriptional function. Interestingly, thyroid ultrasonography detected a normal thyroid tissue in the patient.

P0757. Haploinsufficiency of the SERPINA6 gene is associated with severe muscle fatigue: First de novo mutation in corticosteroid-binding globulin deficiency

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Rare variants of the corticosteroid-binding globulin SERPINA6 with reduced binding affinity to cortisol have previously been found in very few families. Of these, a 367Asn variant, previously designated “CBG Lyon” by Emptoz-Bonneton and coworkers (2000), was shown to be associated with muscle fatigue in two out of three of the known homozygote carriers. Heterozygote carriers of the “Lyon” variant were not described as affected in these families. However, five out of 19 carriers of the “Null” allele of a previously described Trp12-to-Stop mutation had Chronic Fatigue Syndrome (Torpy et al., 2001). Here, we describe a de novo mutation resulting in the “Lyon” variant of the paternal SERPINA6 allele in a 22 years old patient with severe muscle fatigue. Our data strongly suggest that haploinsufficiency of SERPINA6 due to the Asp367-to-Asn mutation is involved in pathogenesis of muscular fatigue symptoms. No mutation was found in the patient’s maternal SERPINA6 allele, therefore, we cannot rule out that environmental modifiers may contribute to severity of disease in the patient, since his symptoms were frequently induced by stress, and his autonomic and endocrine reactivity to a laboratory stress test was extremely pronounced, suggesting a synergistic action of stress hyper-reactivity and genetic vulnerability.

P0758. Protein expression analysis of natural PROS1 mutations associated with anticoagulant protein S deficiency

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This study was aimed at analysing the mechanism by which different natural protein S gene (PROS1) mutations result in anticoagulant protein S (PS) deficiency and thrombosis. With this purpose 4 mutations associated with quantitative PS deficiency in 3 Spanish pedigrees (143C>G in 5’-UTR and L-27H, 1418delA and M599T) and a neutral variant (R192K) were introduced by site directed

mutagenesis in the wild type PROS1 cDNA cloned in pcDNA3.1. PROS1 wt and mutant constructs were cotransfected with pSEAP in COS7 cells and the level of expression of the recombinant proteins was analysed by western blot of the cell culture media and the cellular extracts. Protein band densitometry was used to quantify PS, which was normalised through SEAP measurements. The results obtained indicated a highly reduced expression of the L-27H and the 1418delA (frameshift and stop at 405) mutations and a reduced expression of M599T in the cell culture media. L-27H and 1418delA were not detected in the cellular extracts. By contrast, 143C>G expression appeared to be higher than that of wild type PS and no differences were found with R192K. From these results we conclude that PROS1 L-27H, 1418delA and M599T mutations cause quantitative PS deficiency by either reduced synthesis or defective secretion of the mutant protein. Normal expression of R192K confirms that this is a neutral PROS1. Increased expression of 143C>G, identified in the same allele as L-27H, should be further analysed.

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P0759. Genomic deletions account for more than 10% of the *FOXL2* mutations in BPES families and can be revealed by MLPA analysis.

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Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, MIM 110100) is an autosomal dominant genetic condition in which an eyelid malformation is associated (type I) or not (type II) with premature ovarian failure (POF). In 2001, mutations in the *FOXL2* gene, encoding a forkhead transcription factor, have been shown to cause both types of BPES. Since then more than 130 intragenic *FOXL2* mutations have been reported and collected in the Human *FOXL2* Mutation Database (http://medgen.ugent.be/foxl2/).
In our series of BPES patients we identified 80 *FOXL2* rearrangements, including 71 intragenic mutations, 1 balanced reciprocal translocation and 8 *FOXL2* deletions. Two of these are novel partial *FOXL2* deletions occurring in sporadic BPES patients, one a novel total gene deletion in a BPES type I family and five are microdeletions of the *FOXL2* region, three of which are novel. None of these deletions have been found in unaffected individuals.
For the detection of the deletions we applied a combined approach of custom-made MLPA (Multiplex Ligation-dependent Probe Amplification, MRC-Holland), FISH analysis and genotyping with more than 30 microsatellite repeat markers.
In conclusion, this is the first study demonstrating the involvement of partial and total gene deletions of *FOXL2* in the causation of BPES. These deletions can be revealed by MLPA, being a novel tool for *FOXL2* gene dosage analysis. These results show that genomic deletions account for more than 10% of the *FOXL2* mutations in BPES families and underline the importance of *FOXL2* gene dosage analysis in routine screening of clinically well-defined BPES patients.

P0760. Cellular mislocalization of mutant ALADIN causes triple A syndrome

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The triple A syndrome (MIM*231550) is a rare autosomal recessive disorder characterized by adrenal insufficiency, achalasia of the cardia, alacrima, and a variety of neurological and dermatological features. This disorder maps to chromosome 12q13 and is caused by mutations in the *AAAS* gene encoding a novel WD-repeat protein called ALADIN. ALADIN localizes to nuclear pore complexes (NPCs), which is the sole site of nucleocytoplasmic transport. Investigating 98 families with triple A syndrome mutation hotspots became apparent including Q15K (exon 1) and S293P (exon 8) occurred in 16 respectively 21 families from different regions. Genotype/phenotype analyses revealed a highly variable occurrence, age of onset and severity of all clinical symptoms between patients with the same mutation. The severity and progression of neurological features cannot be correlated to the localization and the nature of the mutations in ALADIN. To examine the cellular localisation of wildtype and mutant ALADIN we investigated nine different ALADIN mutants: two nonsense (W84X, Q456X), two frameshift (F157fs, G397fs) and five point mutations (Q15K, L25P, H160R, S263P, L381R) using EGFP- (enhanced green fluorescence protein) tagged fusion proteins. Whereas the proteins nonsense mutations were found in the cytoplasm and the nucleus the proteins bearing frameshift and 4 out of 5 point mutations were mislocalized predominantly in the cytoplasm. Despite the fact that the Q15K mutant protein does not affect NPC-targeting, patients bearing this mutation exhibit a moderate to severe phenotype similar to those with truncating mutations. This supports the hypothesis that ALADIN may play an important role in nucleocytoplasmic transport.

P0761. Frequency of ocurring of the 3849+10kb C>T mutation in Polish CF patients is significantly higher than in most of other populations.

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The 3849+10kb C>T mutation belongs to the group of splicing CFTR gene defects. It creates a partially active splice site in intron 19 that can lead to the insertion of a new 84-bp cryptic exon containing an in-frame stop codon. This mutation has been found to be associated with a milder form of disease manifestation. Patients carrying this mutation in one allele are characterised by: better nutritional status, older age of onset, normal or slightly elevated chloride values (in comparison to homozygotes for delF508 mutation).
Till now there have been 30 CF patients genotyped in our laboratory who have 3849+10kb C>T mutation in at least one allele of CFTR gene. There are 26 patients in this group with genotype: 3849+10kb C>T / delF508. There are also two patients who are homozygotes for 3849+10kb C>T mutation, whose clinical data are being currently collected. They are members of two unrelated families. This genotype is very rare in Poland and in the world. In case of the other patients second mutation has been also identified.
5 patients are carriers of this mutation in one allele and any other mutation in second allele has not been found.
Mutation 3849+10kb C>T is found in Polish population with frequency of 3,9% of CF alleles which places this mutation at second position after delF508 (53%).
In comparison to the frequency in world population (0,2%) it`s significantly higher score. That is why the identification of this mutation is a part of the routine diagnostics provided by our laboratory.

P0762. Novel mutations in the GJB1 - connexin 32 (Cx32)-gene in Czech CMTX patients.

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Charcot-Marie-Tooth disease (CMT) is the most common hereditary neuromuscular disorder characterised by distal muscular weakness and atrophies, gait abnormalities and sensory deficit resulting from the peripheral neuropathy. CMTX1 is the second most frequent genetically defined X-linked dominant subtype of CMT, caused by mutations in the gap junction beta 1 (GJB1) gene.
We report four, not yet reported mutations in the GJB1 gene (Leu9Phe, Val63Phe, Ile127Ser, Leu212Phe) detected in Czech CMT patients. All reported mutations segregated with the CMT phenotype. These mutations affected the intracelullar domains of the GJB1 protein - 1. IC, 2. IC and 3. IC respectively. In the families with mutations Leu9Phe, Val63Phe, Ile127Ser, women were substantially milder affected than men and their age of onset was as least 20 years

later than in men. Affected men presented often by severe muscle atrophies in the four and fifth decade. Nerve conduction velocities (NCV) in affected patients were mostly within 30-40 m/s range and often were ranged as intermediate or axonal lesion. Hearing loss was not reported from any of these families.
The mutation Leu212Phe appears to be de-novo, which is very rare in GJB1, since it was not detected in the patient´s mother. Unfortunately this family was no more available for further studies. GJB1 gene should be tested in all CMT patients with an X-linked dominant family tree without the CMT1A duplication regardless to the electrophysiological type of polyneuropathy. Mutations in the GJB1 are more probable in familiar cases than in sporadic cases.
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P0763. Mutation analysis in Danish families with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a complex genetic syndrome resulting in retinal dystrophy, postaxial polydactyli, obesity, hypogenitalism among males, renal manifestations, and learning disabilities. Linkage studies show great genetic heterogeneity. 8 loci have been identified, and 6 genes have been cloned. Until recently, BBS was thought to be an autosomal recessive disease, but it has now been shown that in some cases the disorder shows triallelic inheritance.
Of 100 patients with Bardet-Biedl syndrome registered in the “Retinitis Pigmentosa Register” at the National Eye Clinic for the Visually Impaired (Copenhagen, Denmark) 60 patients in 53 families gave accept to participate in our research. The aim of the project is to genotype the Danish Bardet-Biedl population. In BBS1 one frequent mutation has been identified, M390R, with a frequency of 18-36 % among all BBS-patients (allele frequency 0,14-0,32). We tested for this mutation by restriction enzyme analysis of a pcr product. We found 10 homozygotes (9 families) and only one heterozygote, corresponding to19% of the Bardet-Biedl patients having this mutation (allele frequency 0,18).
Mutation analysis is being performed of the BBS1 and BBS6 genes using dHPLC, denaturing high performance liquid chromatography. Results from the mutational analysis will be presented.

P0764. Two novel mutations in the FGD1 gene in Aarskog-Scott syndrome

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Faciogenital dysplasia 1 (FGD1) gene has been identified as a responsible gene for Aarskog-Scott syndrome (AAS). We found two novel point mutations of the FGD1 gene in two AAS families. In one family, a missense mutation was identified in the exon 11 (2626C>T), substituting tryptophan for arginine at the position 636 whithin the pleckstrin homology domain of FGD1. In the second family, a nucleotide transition was identified at the first position of 5’ splice site of intron 14 (IVS14+1G>A), reducing FGD1 expression. This is the first report of a mutation at a splice site of FGD1 gene.

P0765. A mitochondrial DNA mutation (A3243G mtDNA) in a family with cyclic vomiting.

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Patients with a cyclic vomiting syndrome (CVS) have experienced a minimum of three distinct episodes of vomiting and nausea usually involving more than four emeses in one hour at the peak. They feel quite well between episodes.
A family was recruited with four members suffering from CVS: a 5 year old boy , his mother, the maternal grandmother and aunt. The three adults were affected by CVS during childhood. Metabolic investigations revealed permanent hyperlactemia with elevated L/P in the boy‘s mother. In the maternal grand-mother and aunt, lactate levels were slighthy elevated, and L/P molar ratios remained in the normal range.
The young patient suffers from vomiting fits which last for many hours until spontaneous resolution and recur with the same characteristics after an interval of 15-20 days. Metabolic acidosis with hyperlactemia and elevated L/P and ketone body molar ratios were found. Hyperalaninemia, lactaturia, and abnormal excretion of suberic, adipic, and 3-hydroxy-butyric acids were observed.
Screening for 3243 np mutation in mitochondrial DNA was carried out by PCR-RFLP method.
The A3243G bp mutation was detected in the boy and in his above mentioned relatives. In the boy’s blood, the 3243 bp mutation with 70% mutant mtDNA was detected. The mtDNA in the muscle was heteroplasmic for the 3243 bp mutation in all cases.
Our study has pointed out that in cases where the CVS appears to show maternal segregation, particularly when it is associated with increased lactates, mtDNA may be a suitable subject for further investigation.

P0766. Congenital afibrinogenemia caused by uniparental isodisomy of chromosome 4 containing a 15-kb deletion involving fibrinogen Aα-chain gene

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Uniparental disomy (UPD), i.e. the inheritance of both copies of a chromosome from only one parent, can occur either as heterodisomy or isodisomy. Several mechanisms (gametic complementation, trisomic rescue, and compensatory UPD) may lead to UPD, which can result in clinical condition either by producing homozygosity for recessive mutations or as a consequence of aberrant patterns of imprinting. To date, out of the 47 theoretical possibilities of UPD for entire chromosomes, 32 have been observed. Their highly variable frequency depends on the involved chromosome and on its parental origin. Concerning chromosome 4, only one case of UPD for maternal isochromosome 4p and 4q has been described in a living patient.
In the last few years, several genetic defects have been identified in the fibrinogen gene cluster, located at 4q31.3-q32.1 chromosomal region, as the cause of congenital afibrinogenemia. This rare autosomal recessive coagulation disorder is characterised by the lack of fibrinogen in plasma.
In this study, a mutational screening performed in an afibrinogenemic Thai proband enabled the identification of a homozygous 15-kb deletion involving the fibrinogen Aα-chain gene that represents the largest afibrinogenemia-causing deletion identified so far. Since this mutation was inherited only from the mother and non-paternity was rule-out, a maternal UPD was hypothesised. Genotyping of family members for markers spread over the entire chromosome 4 revealed a maternal isodisomy.
The absence of pathological phenotypes other than afibrinogenemia in the analysed proband suggests that there are no maternally imprinted genes on chromosome 4 with a major effect on phenotype.

P0767. Mutation analysis and clinical course in NF2 Polish patients

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Neurofibromatosis type 2 (NF2) is an autosomal dominant disease of the nervous system characterized by a tumor predisposition in which affected individuals develop multiple schwannomas, meningiomas and ependynomas. *NF2* gene has been hypothesized to function as a tumor suppressor gene. We were looking for mutations in *NF2* gene in 23 patients (17 families) with clinical features of NF2, sending from different medical centers in Poland. Using SSCP as a screening method for detection of mutations in blood and tumor samples and sequencing DNA we have found 5 germline nonsense mutations in our tested group. Three of these mutations are known: c.52 C.T in exon 1; c.169 C.T in exon 2; c.592 C.T in exon 6. Two mutations in exon 11 are new: c.1002_1003insG and c.1029_1030insCC. After detailed clinical analysis we conclude that all of these defects

correlate with severe clinical course (they generate truncating protein-product). What is very interesting that we did not find any somatic mutations in tested tumor samples suggesting that there are may be any other mechanisms, except of Knudson's two hit hypotesis, which are involved in expression of *NF2* gene. Clinical observations of our patients are consistent with D.Evans's group results (2002) concerning mortality of NF2 patiens and recommendation that they could be reffered to specialty treatment centers.

P0768. Study of the involvement of the Fragile X Syndrome molecular mechanisms on the clinical characteristics of carrier females: Preliminary Results

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Fragile X syndrome (FXS) is the most common inherited cause of mental retardation. Although the clinical phenotype is well known in males, this is not the case in carrier females.
We have undertaken a complete clinical and molecular study in carrier females and in normal women belonging to FXS families ascertained in our Hospital with two objectives: 1) to determine the relative impact of the molecular mechanisms of FXS -number of CGG repeats, methylation status, gene expression and FMRP production- on the clinical involvement of female carriers and 2) to study which of these parameters could predict better the phenotype in females to improve diagnosis, and prevention.
We present here our preliminary results in 37 females belonging to 15 different families. CGG expansion and methylation were studied by PCR and Southern blotting (Stb12.3), obtaining 10 FM, 1 Mosaic, 19 PM and 7 normal. We have determined the relative levels of FMR1mRNA by use of quantitative real-time fluorescence detection (RT-PCR) in 20 females, showing only a slightly overexpression in PM women. Finally, we have studied the FMRP expression in hair roots by antibody test in 28 females, showing a very low percentage of expression in FM females (between 5 to 10%).
Physical and behavioural phenotype has been completely studied in the 37 females: In FM women, 6/10 have the typical FXS phenotype, and mental retardation in different degrees is present in 7/10 of them. Most of the studied PM carrier females have adaptive disorder and four of them present ovarian failure.

P0769. Reverse-hybridization assay for mutations associated with hereditary sugar intolerance

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Dietary carbohydrates for humans include polysaccharides (starch), disaccharides (sucrose, lactose) and monosaccharides (glucose, fructose, galactose). During digestion, specific enzymes will initially hydrolyze poly- and disaccharides into their monosaccharide constituents, which then become absorbed by apical cells of the small intestine and further metabolized. A variety of genetically determined enzyme and transporter deficiencies may cause hereditary intolerance to common dietary sugars. Lactose intolerance (adult-type hypolactasia, lactase non-persistance) is an extremely frequent autosomal recessive condition causing diarrhea, nausea and flatulence. It is highly associated with two mutations located upstream from the lactase-phlorizin hydrolase (LPH) gene locus. Hereditary fructose intolerance is an autosomal recessive disorder caused by mutations in the aldolase B gene. Affected subjects suffer from severe abdominal pain, vomiting, hypoglycaemia, and unless fructose-containing food is strictly avoided may even die from irreversible damage of the liver and kidney.
We have developed a reverse-hybridization assay for the rapid and simultaneous detection of two mutations (-13910 C/T, -22018 G/A) upstream to the LPH gene and four mutations (del4E4, A149P, A174D, N334K) in the aldolase B gene. The test is based on multiplex DNA amplification and hybridization to a teststrip presenting a parallel array of allele-specific oligonucleotide probes for each mutation. 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 test is simple and convenient, requires very small amounts of samples, and can easily be modified to include additional mutations.
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P0770. Analysis of the proteolipid protein gene in patients with PMD

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Pelizaeus-Merzbacher disease (PMD) is a rare hypomyelinating disorder of the central nervous system with X-linked recessive inheritance. The prevalence in the US is estimated to be about 1/300,000-1/500,000. Clinical features include nystagmus, ataxia, stridor, spasticity, and mental retardation. The clinical severity and age of onset vary widely among the patients. About 80% of patients clinically diagnosed as PMD have been shown to carry a mutation in the proteolipid protein (*PLP*) gene, which is located on chromosome Xq21.3-Xq22. In addition to the point mutations and deletions in the *PLP* gene, duplications involving the entire gene have been shown to be the major genetic abnormality causing PMD.
In the framework of this study, the genetic basis of PMD was investigated in samples from 16 PMD families, including a total of 20 patients (16 males and 4 females). None of the male patients in our cohort showed heterozygosity for RFLP markers, exon 4-*Aha*II and DXS17-*Taq*I polymorphisms within the *PLP* gene. Interestingly, the affected brothers in one family and the sisters in another were found to inherit different maternal alleles for at least one of the markers used, suggesting genetic heterogeneity. To detect duplications interphase FISH and quantitative multiplex PCR are currently being performed. Screening of the *PLP* gene in these patients using SSCP and subsequent sequencing analyses revealed presence of exon 4-*Aha*II polymorphism in five patients and a previously reported P215S mutation in hemizygous condition in another patient.
The present study will help our understanding of the pathogenesis of the PMD phenotype.

P0771. Frequency of *HFE* H63D, S65C and C282Y mutations in patients with iron overload and controls from Toledo, Spain.

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Hereditary Hemochromatosis (HH) is an autosomal recessive disease caused by a defective iron absorption. C282Y is the most frequent *HFE* gene mutation causing HH in Northern European populations and their descents. However, two other mutations, H63D and S65C, have been described as pathogenic changes. In this study we have tried to evaluate the frequency of these three mutations in our community. Ninety three patients and one hundred fifty controls were screened for H63D, S65C and C282Y mutations using a PCR-RFLP based strategy. We have found a very low frequency of C282Y homozygous patients 7% in disagreement with previous studies. The remaining patients were H63D homozygous 17%, H63D/C282Y compound heterozygous 9%, H63D/S65C compound heterozygous 1%, H63D heterozygous 24%, C282Y heterozygous 8%, S65C heterozygous 2% and 32% of patients lacked any of the 3 mutations studied, despite they showed clinical/biochemical Hemochromatosis features. We have observed a high frequency of H63D mutation both in controls and patients and the main genotypes implicated in HH in our series were H63D homozygous and H63D/C282Y compound heterozygous. We propose here, do not avoid the H63D mutation study in HH patients from our geographic area. Moreover, further studies are needed to elucidate what is the role of this mutation in the developing of HH and what genetic/environmental additional factors are implicated in it.

P0772. Ten years of study of PWS Polish population

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The Prader-Willi Syndrome (PWS) is a genetic and neurologic behavioral disorder linked to abnormalities in imprinted domain on chromosome 15q11-q13, characterized inter alia by hypotonia, hyperphagia in early childhood resulting in obesity, hypogonadism, short stature and moderate mental retardation. All of genetic abnormalities in PWS are associated with loss of expression of paternally derived alleles. Using karyotyping, FISH and methylation analysis we have examined 292 patients, diagnosed in different medical centers in Poland, susspected to be PWS and we confirmed PWS diagnosis in 102 cases (35%). We identified 51 cases of paternal deletion (50%), 16 patients with mUPD (16%) and 3 patients with imprinting defect (3%). In 5 cases we could not detect molecular defect because of noninformative microsatellite analysis. All of PWS patients have classical phenotype for deletion, mUPD and ID what we present in details. Four of patients of our 292 cohort have shown PWS-like phenotype but we did not find any abnormalities on chromosome 15. Because of PWS is a multigenic disorder it is possible that gene mutation may occur in these patients. We also conclude that there is significant difference between correct clinical diagnosis of PWS in specialty treatment centers then in others. In specialty treatment Polish medical centers the propotion of confirming PWS and sharing of particular molecular defects are consistent with those observed in tested cohort in other caucasian population.

P0773. Detection of subtelomeric rearrangements in patients with idiopathic mental retardation using MLPA

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Despite the clinical relevance of subtelomeric rearrangements, responsible for 5-10% of cases of unexplained mental retardation, methods to screen for cryptic chromosome aberrations near the telomeres on a routine basis are scarce. None of the methods described so far are entirely satisfactory, e.g. fast, reliable and cheap. Multiplex Ligation-dependent Probe Amplification (MLPA) is a new method based on the amplification of ligated probes hybridized to chromosome ends. It enables detection of possible rearrangements of all chromosome ends in just two multiplex reactions. Our initial study revealed six subtelomeric rearrangements in 126 patients with idiopathic mental retardation, including two 1pter deletions, a 1qter deletion, a 3pter deletion, a der(11)t(11;20)(qter;qter) and a 19pter duplication, giving a detection rate of 4.8%. In one case, an apparent 21qter deletion appeared to be a false positive due to a 3bp deletion at the site of the probe. Recently a new probeset in which all telomeres can be screened in just one multiplex reaction was developed. We were able to detect all tested aberrations with this novel probe set. Moreover, results for chromosome ends 2q and 6p that were variable in the first generation probe set, were more reliable.

In addition, we have narrowed the breakpoint region of the patients with the terminal 1p, 1q and 11q deletions down to 30-68 kb and aim to determine the breakpoint sequence in order to increase our insights in the mechanisms responsible for the telomeric chromosome breakage. Moreover, we aim to survey the genes involved in the clinical phenotype.

P0774. Analysis of Protein expression level in Two-Dimensional Gels of Fragile X Syndrome

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Fragile X syndrome is the second most common genetic cause of mental retardation. It is caused by a large expansion of this CGG repeat (full mutation) that leads to silencing of the FMR1 gene and the absence of the FMR1 gene product, a 70 - 80 kDa protein (FMRP). The aim of this study is to qualify and quantify the expression level of the proteins separated in two dimensional gel analysis. Two-dimensional polyacrylamide gel electrophoresis (2D PAGE) is the only method currently available for the simultaneous separation of thousands of protein according to their isoelectric point and molecular weight. In this study, protein expression maps for sera from healthy individuals are compared with maps from patients with fragile X syndrome. PDQuest™ analysis detected 75 % of proteins being conserved between the normal and fragile X serum which are most likely the housekeeping proteins of the samples. Two protein spots of interest and another four protein spots are found to be 5 fold down-regulated in fragile X patient serum. There are also two protein spots that are over expressed in fragile X syndrome serum. Further studies should be carried out using proteomics approach in attempt to explain the structural, functional and interactive properties of FMRP protein in serum towards gene therapy and drug development purposes.

P0775. The patient with Duchenne`s muscular dystrophy: case report

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Duchenne`s and Becker`s muscular dystrophy (DMD & BMD) is an X linked recessive lethal disease, that represent the most common genetic neuromuscular disease of childhood.

Sixty five percent of DMD and BMD cases are caused by deletion of one or more exons in the dystrophin gene, duplications cause these diseases in 6% to 7% of the cases.

Our patient is a 7-years-old boy referred to the geneticist with following indications: muscle weakness, difficulty climbing stairs, celiac disease, and undifferential hepatitis. The proband is the fourth child of healthy, unrelated parents.

The diagnostic investigations showed the elevated level of serum creatine kinase, AST and ALT.

The molecular diagnostic for this patient was carried out by multiplex DNA amplifications of the dystrophin gene using three multiplex PCR assays: the 9-exon Chamberlain-set, 9-exon Beggs-set and 7-exon Kunkel-set. There were found 15 deletions of different exons in the dystrophin gene.

Because of so large deletion in dystrophin gene the deletion in contiguous glycerol kinase gene is presumable, and glycerol kinase enzyme activity should be measured. Thereby, this case requires the further investigation.

P0776. New mutations and polymorphisms identified in MECP2 gene causing Rett syndrome

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Methyl-CpG-binding protein 2 (MeCP2) is a protein which binds specifically to methylated DNA and is thought to act as a global transcriptional repressor. MeCP2 contains two main functional domains: a methyl-CpG-binding domain (MBD) followed by a transcriptional repression domain (TRD). Mutations in MECP2 gene lead to decrease/loss of MeCP2 function and cause a severe neurodevelopmental disorder Rett syndrome (RTT). It affects almost exclusively females and is primarily sporadic in nature. More than 200 mutations have been identified in MECP2. We report mutation analysis of 58 girls with clinical diagnosis of RTT from Czech and Slovak Republics. Genomic DNA was used to amplify the coding sequence and exon/intron borders of MECP2 gene. Products were examined by RFLP and sequencing. The analysis revealed 20 different mutations in 48 patients (82%). Four of them have not been previously published: a missense mutation P302S and deletions IVS2-16delT, S357fs and ΔS357. Moreover the frequency of mutation T158M compared to other databases (9.34%) has been much more higher in our patients (20.83%). Two novel polymorphisms - 587C>G and 1161C>T were also detected. Our results show molecular heterogeneity in Czech and Slovak patients with RTT, facilitate the

RTT diagnosis at the molecular level, and provide insight into the molecular pathology of RTT. Supported by grant LN 00A079.

P0777. Various novel patterns of inheritance of the long QT syndrome: Pitfalls in molecular genetic diagnosis.

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P0778. Association of the neprilysin (NEP) gene with susceptibility to complex regional pain syndrome (CRPS)

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P0779. VSX1 mutational analysis in keratoconus

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Keratoconus is a frequent non inflammatory disorder characterised by progressive conical protrusion of the cornea and central stroma thinning. It is a major indication for cornea transplantation in the Western world and its prevalence is 1:2000. Although the vast majority of cases are sporadic, a positive family history has been documented in 6-10% of patients. The autosomal dominant pedigrees are more frequent and strongly reveal incomplete penetrance and variable expressivity. In these families the astigmatism is interpreted as fruste form of keratoconus. Mutations in *VSX1* homeobox gene have been identified in patients affected by two distinct inherited corneal dystrophies, posterior polymorphous dystrophy and keratoconus.

Direct sequencing of the five exons and intronic flanking regions of the *VSX1* gene in 63 Italian patients, diagnosed by corneal topography, allowed us to identify three nucleotide changes leading to the, already described, aminoacid substitutions D144E, G160D and P247R. The changes were detected at heterozygous state in all individuals identified. The D144E allele was found in a patient and in his son affected by astigmatism. The G160D and P247R alleles were found either in affected and clinically unaffected individuals in two keratoconus families.This observation is in agreement with the concept accepting the keratoconus as a dominant trait with variable expression and incomplete penetrance. Nevertheless, considering the keratoconus not strictly as a mendeleian trait but to some extent a complex disease, the previous and our results could represent a dowel of an interesting puzzle that is being clarifying.

P0780. The Gordian knot is cut: multiplex ligation-dependent probe amplification allows reliable identification of female DMD/BMD carrier

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A long standing problem in human genetics and especially in diagnostics is the fast and reliable identification of changes of gene copy numbers. A well known example is the Duchenne muscular dystrophy. It is known since about 15 years that in 60% of the patients deletions within the dystrophin gene are disease causing. However the identification of female carriers remained a difficult analytical challenge and identification of exon duplication events which are disease causing as well, have been that far out of range that until recently even the frequency of this type of rearrangements was completely unknown. A couple of techniques have been developed to circumvent these problems (real time PCR, multiplex amplifiable probe hybridisation MAPH), but it turned out that these techniques are too complicated or uncertain to perform diagnostics in a routine setting.

By analysis of 20 so far unresolved DMD/BMD cases we proved that the recently developed multiplex ligation-dependent probe amplification technique (MLPA; MRC-Holland) is sensitive enough to uncover all female carriers of deletions and duplications. Reliability of the results is exceptional, automation is possible and we developed an Excel based program to facilitate calculation of the results. After loci or gene specific adaptation MLPA technology enables reliable determination of gene copy numbers in the complete genome. Even analysis of genomic DNA isolated from archived tissue specimen is possible and we expect that use of this technique will be helpful to answer many yet unresolved questions in human genetics.

P0781. GJB2 gene mutations: genotypic and phenotypic correlation

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GJB2 mutations represent the first cause of congenital non-syndrommic hearing loss in developped countries (DFNB1). We analysed the phenotype and the genotype of 265 French patients presenting a homozygote mutation or double heterozygote mutations in GJB2, or the association of a GJB2 mutation with the GJB6 deletion : del(GJB6-D13S1830). If the 35delG remains the more frequent variant of GJB2 (68% of the alleles), we identified a total of 29 different mutations in this gene, and 6 of them could be considered as recurrent. We described 4 new mutations 355del9, 573-574delCA, M151R and Y115X. We have compared the deafness observed in patients homozygous 35delG and in patients with other genotypes. The mutations L90P and V37I were associated with a less severe phenotype than homozygous 35delG. This result is important for genetic conselling and physiopathologic studies of DFNB1.

P0782. The mutational spectrum of *RUNX2* and genotype-phenotype correlations in cleidocranial dysplasia (CCD)

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Cleidocranial dysplasia (CCD) is a rare bone dysplasia characterized by high penetrance and variable expressivity. Mutations in the Runt-related transcription factor *RUNX2* have been correlated with this disorder. Several mutations affecting the coding region of this gene are known.

The aim of this study was to identify the spectrum of mutations in the *RUNX2* gene in our CCD patients' cohort and to determine possible genotype-phenotype correlations. Since most of the symptoms are located in the craniofacial region, the phenotype was examined focusing on craniofacial and otorhinolaryngological aspects. Therefore, radiologic and clinical examinations were carried out under defined conditions. After informed consent, the coding sequence of the *RUNX2* gene was analyzed using PCR, restriction digest and direct sequencing.

We screened the *RUNX2* gene for mutations in 26 familial and sporadic patients and identified 11 different mutations in a total of 19 patients, including 13 index patients. In total we detected 5 different missense mutations in 7 subjects, i.e. in 54 % of the 13 index patients, and 4 frameshift mutations, as well as 2 stop mutations. The overall CCD phenotype as defined by our clinical observations varied from mild expressions to the full blown phenotype.

A clear intrafamilial variability as well as a clear intraphenotypic correlation was found. Furthermore, a clear correlation between the overall expressivity of the phenotype and the localization of the mutation within the RUNX2 protein was established. This study gives

new insights on the craniofacial correlation of the phenotype to the genotype involving a wide interdisciplinary approach.

P0783. A new ESE (Exonic Splicing Enhancer) mutation as cause for recurrent infections

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One of the major mechanisms of the innate immune system to prevent infections of the body with pathogenic microorganisms is the activation of a system of plama proteins, the complement, which leads to convertion of the complement components C3 and C5 into their biologically active split products. Dysfunction of C5 results in propensity for severe recurrent infections. Up to now only two nonsense mutation for human C5 deficiency have been reported. Here we report a patient with complete C5 deficiency as revealed by immunochemical and functional analyses. No obvious functional mutation was found after sequencing the 41 Exons of the gene (including the promotor region), only a nucleotide transition in Exon 10 leading to an exchange from one alkaline amino acid to another one. Family members with reduced serum C5 were heterozygous for this sequence alteration whereas the patient was homozygous. We suggsted that this alteration is causative or is tightly linked to the causative mutation. Recent findings revealed, that exonic splicing enhancers (ESEs) facilitate the recognition of an exon by the splicing apparatus and a mutation in an ESE could result in splicing defects. To determine the possible impact of this transition on an ESE we used the Rescue-ESE programm. The result showed that the nucleotide exchange alters and shifts but does not abolish an ESE. RT-PCR experiments with RNA from lymphocytes of all family members showed skipping of Exon 10 in all individuals with the mutated allel leading to a frameshift and a premature stop codon.

P0784. Molecular Step Diagnostics of Long QT Syndrome

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Long QT syndrome (LQTS) is a disorder of the cardiac conduction system that is characterized by a delay in repolarization and prolongation of the QT interval >440 msec. Affected individuals are predisposed to ventricular tachyarrhythmias (torsades de pointes), syncopes, seizures and sudden death. Five genes coding for ion channel proteins have been implicated in the autosomal dominant Romano-Ward form of LQTS: KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2. Mutations in KCNQ1 and KCNE1 may also cause the recessive Jervell Lange Nielsen form that is associated with deafness and a QTc >500 msec. Some mutations in the SCN5A gene result in Brugada syndrome, a form of idiopathic ventricular fibrillation characterized by right bundle-branch block pattern, ST segment elevation and sudden death.
Molecular diagnostic analysis of all 5 LQTS-associated genes is labour intensive and costly. We therefore developed an efficient double stranded sequencing based mutation scanning method. The analysis is performed in three steps taking mutation frequency in the different exons into consideration. To date, 170 unrelated individuals with LQTS were analysed step by step. More than 100 different mutations were found in 110 independent families. 60% of the mutations resided in mutational hot spots such as exons coding for the pore regions and were identified in the first step (9/62 exons).

P0785. Mutations in Norwegian Cystic Fibrosis Patients.

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The Norwegian CF mutation frequencies are missing from the European CF map.
In Norway we have registered ca.250 patients with Cystic Fibrosis. We have determined CFTR-mutations in CF-patients referred to our lab for the last 10 years. Our Norwegian patients come mainly from the eastern and middle part of Norway. Using the ABI OLA 31m mutation kit and 3 additional mutations (394delTT, R117C and 4005+2T-C) we have determined the genotype in 151 patients. We then sequenced all 24 exons in the CFTR gene with the intron boundaries in patients with one confirmed mutation. The second mutations were found the in 23. The mutation frequencies found in our patients are presented

P0786. Novel CYP11B1 sequence variants in adolescent and adult patients with clinical signs of hyperandrogenism

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Steroid 11ß-hydroxylase (*CYP11B1*, P450c11) is a mitochondrial cytochrome P450 enzyme necessary for cortisol biosynthesis. Point mutations, small insertions and deletions as well as complex rearrangements have been identified in patients with classic and non-classic 11ß-hydroxylase deficiency.
We sequenced exon and flanking intron sequences as well as parts of the 5'- and 3'-flanking regions of *CYP11B1* genes in 490 unrelated patients from different ethnic origin with the clinical diagnosis of hyperandrogenism. Androgen excess due to mutations in *CYP21* was excluded prior to screening *CYP11B1*. Apart from previously reported polymorphisms, 24 novel sequence variants were detected in *CYP11B1*. Six variants (-225G>A, -216A>G, -196_-186del(GGA $)_3$GGins(GGA$)_2$GG, -179_-178delCCinsC, -170C>T, -150C>G) are located in the 5'-flanking region. Five silent mutations in codons 81 (TAC>TAT), 145 (AAT>AAC), 338 (CAG>CAA), 346 (GCC>GCT) and 347 (GCC>GCG), respectively, as well as 8 missense substitutions, L24R (CTG>CGG), D82N (GAC>AAC), V129L (GTG>CTG), P135T (CCT>ACT), S150L (TCG>TTG), A415T (GCC>ACC), G452R (GGG>AGG) and D480N (GAC>AAC) respectively, were detected in the coding region. The nonsense mutation Q102X (CAG>TAG) was found in exon 2. Finally, we identified the intronic substitutions IVS2+6C>T, IVS2-58G>A, IVS2-57G>T and IVS2-42C>T, respectively. Most variants (21/24) were observed only once or twice. The presence of -216A>G and IVS2-42C>T (allele frequency 1.7% and 2.1%, respectively) with similar frequency in a healthy control group suggests that both variants are non-pathogenic polymorphisms. While silent mutations should not affect 11ß-hydroxylase activity, the clinical importance of the majority of the described changes in non-coding and coding regions of *CYP11B1* has yet to be defined.

P0787. Lymphocyte culture with puromycin treatment improves NF1 mutation screening efficiency

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The analysis strategies for Neurofibromatosis type 1 (NF1) diagnostics have continuously evolved in function of the increased knowledge of the NF1 gene and the new molecular techniques available in our field. Since 1991 we have been offering genetic testing for Neurofibromatosis type 1 (NF1) via mutational screening of the entire NF1 coding region using the cDNA-SSCP/HD approach, with all alterations being characterised at both the DNA and RNA level. Although this method dramatically increased the mutation detection rate, a considerable percentage of mutations remained undetected. Apart from the inherent difficulty due to the large size of the gene and the great heterogeneity of mutations, the main technical problem was the generally poor quality of RNA obtained due to inevitable delays in transporting blood samples to our laboratory. This leads to two major problems, RNA obtained from aged samples shows a high number of artefacts due to a cold-shock effect and the detection of germline nonsense mutations can be hampered by the NMD phenomenon. In order to avoid these problems, and as reported by others (Wimmer et al. 2000), we have introduced short-term lymphocyte culture with puromycin treatment prior to RNA extraction. These cultures have lead to a great improvement in RNA quality, the virtual exclusion of artefacts and the elimination of the NMD event. Although results are very preliminary, we have improved our detection rate from 65% (81% familial/51% sporadic) to 86% (100% familial/82% sporadic), together with a significant reduction in the time and costs required for performing the screening.

P0788. The Influence of *FBN1* mutations on Fibrillin-1-Versican Binding

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The Marfan syndrome (MFS) is a common hereditary disorder of connective tissues that is characterized by clinically highly variable manifestations in the cardiovascular, skeletal, and ocular systems. MFS is caused by mutations in the fibrillin-1 gene (*FBN1*) on chromosome 15q21.1. Fibrillin-1 is a main component of the 10-12 nm microfibrils that play an important role in the deposition of tropoelastin during the formation of elastic fibers and also have an anchoring function in many tissue types.
Next to nothing is known about the influence of *FBN1* mutations on the interactions of fibrillin and fibrillin-containing microfibrils with other components of the extracellular matrix (ECM). However, disruption of the intermolecular interactions in connective tissue could be an important aspect of the molecular pathogenesis of Marfan syndrome. Recent results indicate that the C terminal region of versican, associates with fibrillin microfibrils, and binds to an unknown site between cbEGF module #11 and cbEGF module #21. We have narrowed down the binding site between the versican CLD domain and fibrillin-1 using a series of recombinant fibrillin-1 and versican constructs using blot-overlay and other assays. Using *in vitro* mutagenesis, we have generated a series of fibrillin-1 constructs harboring mutations identified in persons with Marfan syndrome and used these to identify the influence of the mutations on fibrillin-1-versican binding *in vitro*. We will present current results on binding studies and on fibrillin-1 and versican immunohistochemical studies of dermal fibroblast cultures from individuals with Marfan syndrome.

P0789. Novel PANK2 mutations and genotype-phenotype correlation in a large collection of european patients with pantothenate kinase-associated neurodegeneration

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Pantothenate kinase-associated neurodegeneration (PKAN) is a juvenile onset, autosomal recessive disease associated with progressive movement alterations, such as dystonia, rigidity, choreoathetosis and spasticity and the less frequent symptoms retinitis pigmentosa, optic atrophy, parkinsonism and epilepsy (MIM 234200). The syndrome is caused by mutations in the *hPANK2* gene which encodes a pantothenate kinase that shows a dual localisation to the cytosol and to mitochondria.
To date 54 mutations have been identified, which are widespread throughout the gene. In this study, we screened for mutations in the hPANK2 gene in 42 unrelated patients of european descent, representing 25 typical and 17 atypical cases. In total, we identified 43 mutated alleles, comprising 24 different mutations and including 14 novel ones. 31 mutations exchanging an amino acid and 12 mutations predicted to result in premature truncation of the PANK2 protein were detected.
A standardized set of clinical data has been compiled from each patient and correlated with the severity of the mutation as assessed (1) according to the degree of evolutionary conservation of the mutated residue and (2) by functional testing of mutant proteins using a complementation assay in *E.coli*. Integrating this data we observe, that the assessed severity of mutations correlates well with the age of onset, but not with the course of the disease.

P0790. Deletion analysis of Macedonian SMA families

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The spinal muscular atrophies are a clinically and genetically heterogeneous group of neuromuscular disorders caused by degeneration of the a-motor neurons of the anterior horns of the spinal cord. According to the age of onset and severity of the clinical manifestations, SMA is classified into three types: Werdnig-Hoffman disease (SMA I) the most severe form, SMA type II with intermediate severity and Kugelberg-Welander disease (SMA III), the mild form. A locus for the three clinical forms of SMA was mapped to chromosome 5q11.2-q13.3. With a estimated birth prevalence of 1 in 10,000 and a carrier frequency of about 1/40 the SMAs are the second most frequent autosomal recessive hereditary disorders.
In this report we present the molecular analysis of the SMN gene in 21 Macedonian SMA families. Deletions of the SMN gene were found in 92% (11/12) of patients with the severe form of SMA, nine of 12 cases were homozygous for a deletion of exons 7 and 8 and two cases were homozygous for a deletion of exon 7. SMN exons 7 and 8 were deleted in 80% (4/5) of patients with SMA type II. We did not find a deletion in four patients with SMA type III. Molecular studies are replacing conventional investigations for SMA and have a high uptake prenatally.

P0791. Molecular genetics of cystinuria: forty-two new mutations, the first SLC3A1 mutation that segregate with phenotype non-I, SLC7A9 mutations that segregate with phenotype I and partial dygenia

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Cystinuria is an autosomal recessive aminoaciduria due to a disorder of renal reabsorption of cystine and dibasic amino acids, that results in urolithiasis of cystine, in which two phenotypes (I: silent heterozygotes, and non-I: amino acid hyperexcretion in heterozygotes) have been described. Mutations in the heterodimeric amino acid transporter for cystine and dibasic amino acid cause cystinuria: mutations in the heavy subunit, rBAT, coded by SLC3A1, cause type I cystinuria while mutations in the light subunit bo,+AT, coded by SLC7A9, mostly cause non-type I cystinuria. 42 new mutations have been identified: 26 in SLC3A1 and 16 in SLC7A9, that include the first two large rearrangements found in SLC7A9. The new and already identified mutations explain 89.9%, 88.2% and 83.8% of the type I, type non-I and untyped alleles, respectively, of a cohort of 166 cystinuria probands. A phenotype-genotype correlation in these families, based on the new genetic classification that we have recently proposed (type A and B due to mutations in SLC3A1 and SLC7A9 respectively) revealed that: i) SLC3A1 mutations show phenotype I with the exception of dupE4-E9 that shows phenotype non-I in most of the cases, and ii) 10 SLC7A9 mutations, which are found in 14% of the type B heterozygotes, show a phenotype I. We also report two mixed families whose carriers bear mutations in both genes, and in one of these families partial non-pathologic dygenia is present as they present higher urinary excretion pattern than the single heterozygotes, but this is not sufficient to cause the disease.

P0792. Molecular genetic diagnosis of calpainopathies: analysis of 20 cases.

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Limb Girdle Muscular Dystrophies (LGMD) constitue a genetically heterogeneous group of hereditary disorders. Clinically, the patients are affected by proximal muscle weakness and wasting, symmetrical and progressive, of upper and lower limbs. Dominant (LGMD1) and recessive (LGMD2) forms are observed. Until now 10 genes have been identified in recessive forms, defining LGMD2A to LGMD2J. LGMD2A is caused by mutations in the CAPN3 gene, located in 15q15.1-15.3. CAPN3 encodes a protein mainly expressed in the squeletal muscle, the calpaïne 3, belonging to a family of intracellular non lysozomal proteases. Its function in the muscular fiber is still not well known, but it could play a role in catabolism and apoptosis, and interacts with other members of the myofibrillar complex (Titine). We are now realizing the molecular exploration of the gene, for diagnosis help, in the molecular genetics laboratory in Marseille. The analysis of the 24 coding exons of the gene has been performed in a first serie of 20 unrelated cases, selected on clinical basis and after Western Blot study of the muscular biopsy. The molecular strategy was based on an SSCP screening followed by sequence analysis of the fragments with an abnormal migration pattern. We thus evidenced 16 mutations, of which only a half have been already reported. We discuss the results in relation to the clinical and proteic data of the patients. As some patients remain with no molecular confirmation of the diagnostic hypothesis of calpainopathy, we also discuss the molecular strategies in this context.

P0793. The brain-expressed c-Jun terminal kinase JNK3 is truncated in a patient with a severe neurodegenerative disorder and associated chromosome translocation

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We have investigated the breakpoints in a male patient with severe neurodegeneration and a de novo balanced translocation t(Y;4)(q11.2;q21). By fluorescence in situ hybridisation, we have identified genomic clones from both chromosome 4 and chromosome Y that span the breakpoints. Fine mapping of the chromosome 4 breakpoint indicated that the c-Jun terminal kinase 3 gene (JNK3) is disrupted in the patient. This gene is predominantly expressed in the central nervous system, and it plays an established role in both neuronal differentiation and apoptosis. Moreover, expression studies in the patient lymphoblastoid cell line confirm that the truncated JNK3 protein is indeed expressed, and that the disrupted transcript is not subject to nonsense-mediated mRNA decay, as is often the case for truncated mRNAs or those harbouring premature termination codons. Over-expression studies with the mutant protein in various cell lines indicate that both its solubility and cellular localisation differ from that of the wild type JNK3; it is plausible, therefore, that the presence of the mutant JNK3 in neuronal cells disrupts normal JNK3 signal transduction, thereby resulting in the severe neurological phenotype in the patient. Current studies aim to characterise the function of both wild type JNK3 and various truncation mutants, and to examine their influences on the cellular localisation of other brain-expressed proteins such as β-arrestin 2 and JSAP1, two of the known JNK3-interacting proteins. These studies will shed light on the mechanism by which the presence of a truncated JNK3 has a dominant and detrimental effect in our patient.

P0794. Choline acetyltransferase (ChAT) and Survival Motor Neurone 2 (SMN2) expression in the spinal muscular atrophy (SMA) spinal cord during human development

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SMA is an autosomal recessive disorder caused by mutations in the SMN1 gene which result in degeneration and loss of motor neurons of the spinal cord. Although the SMN2 gene is the highly homologous SMN1 copy present in all patients, it cannot prevent the disease. Both genes potentially encode for identical proteins although most of the SMN1 transcripts are full length (FL) whereas the majority of the SMN2 transcripts lack exon 7 (delta7). We investigated the expression of ChAT, which is the most specific marker for cholinergic neurons, and SMN2 in the spinal cord of SMA fetuses. SMA spinal cords showed reduced ChAT expression with respect to controls although the proportion of motor neurons negative for ChAT immunostaining was similar in both groups suggesting that the surviving motor neurons in SMA fetuses could have a cholinergic function similar to that of controls. A high level of full-length SMN transcripts with tiny amounts of delta7 isoform (of SMN1 origin) was detected in control fetal spinal cord, whereas SMA spinal cord showed a tiny decrease in the FL but a substantial increase in delta7. Control and SMA spinal cord showed a differential SMN expression whereas in other tissues unaffected by the disease such as intestine, lung, adrenal gland, kidney and eye, the expression was similar between controls and SMA samples. In these tissues, SMN2 may compensate for the absence of SMN1 whereas in SMA motor neurons, the increase in delta7 expression could contribute to the disease. Supported by FIS 02-1275.

P0795. A dual phenotype of periventricular nodular heterotopia and frontometaphyseal dysplasia (PVNH+FMD) in one patient caused by a single *FLNA* mutation leading to two functionally different aberrant transcripts

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Background: Two disorders, periventricular nodular heterotopia (PVNH) and a group of skeletal dysplasias belonging to the oto-palato-digital (OPD) spectrum, are known to be caused by *FLNA* mutations, so far. The cerebral and skeletal phenotypes, however, are considered mutually exclusive because of the different presumed effects of the respective *FLNA* gene mutations, leading to loss of function (PVNH) and gain of function (OPD), respectively. We describe here clinical and molecular findings of the first patient manifesting periventricular nodular heterotopia in combination with frontometaphyseal dysplasia (FMD), a skeletal dysplasia of the OPD-spectrum.
Methods: Molecular analysis was performed by direct sequencing of the entire coding sequence of the *FLNA* gene. Expression of the *FLNA* transcript was studied in fibroblasts by RT-PCR.
Results: A novel *de novo* mutation 7315C→A in exon 45 of the *FLNA* gene was identified. It leads to two aberrant transcripts, one full length transcript with the point mutation causing a substitution of a highly conserved leucine residue (L2439M) and a second shortened transcript lacking 21 bp due to the creation of an ectopic splice donor site in exon 45.
Conclusion: We propose that the dual phenotype (FMD+PVNH) is caused by two functionally different, aberrant filamin A proteins and therefore represents an exceptional model of allelic gain-of-function and loss-of-function phenotypes due to a single mutational event.

P0796. LMNA, MTMR2 and GDAP1 : implication in autosomal recessive Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disorders constitute the most frequent inherited peripheral neuropathies. Electrophysiological criteria serve to classify patients as carrying demyelinating (CMT1), axonal (CMT2) or intermediate neuropathies. All modes of inheritance have been reported, with recessive forms being less frequent, more severe and with earlier onset. We report further evidence that homozygous mutations in LMNA, MTMR2 and GDAP1 cause respectively axonal (CMT2B1), demyelinating (CMT4B1) and mixed (CMT4A) neuropathies in consanguineous families affected with autosomal recessive CMT. We identified the LMNA c.892C>T founder mutation segregating in 11 more Algerian families affected with axonal CMT. MTMR2 sequencing revealed a non sense change and a one-base insertion, respectively in two Algerian families affected with a demyelinating phénotype. Finally, GDAP1 splicing mutation in

one Algerian family and nonsense mutation in a Lebanese family, caused very severe intermediate CMT in both families. LMNA codes for Lamins A/C, major structural proteins of nuclear laminae in vertebrates. MTMR2 encodes a member of the myotubularin phosphatase multigenic protein family, with specific activity towards phosphatidylinositol 3-phosphate. On the other hand, GDAP1 structure and expression pattern locate it at the crossroads between gangliosides and Gluthatione S-Transferases diverse functions. While GDAP1 is mainly expressed in nervous tissues, LMNA and MTMR2 are ubiquitously expressed. Additionally, different mutations in LMNA cause a series of only partially related hereditary disorders. Unraveling the functional interplays underlying the pathophysiology of CMT diseases is today the main issue, focusing on the relation structure-function-phénotype.

P0797. Association of anion exchanger 3 (SLC4A3) gene with idiopathic generalised epilepsy

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Idiopathic generalised epilepsies (IGE) affect about 0.6% of the general population and account for 30% of all epilepsies. The aetiology of IGE is genetically determined, but the complex pattern of inheritance suggests an epistatic interaction of several susceptibility genes. Our recent genome scan on 130 IGE multiplex-families revealed suggestive evidence for a susceptibility locus for common IGE syndromes in the chromosomal region 2q36 (ZNPL = 2.98 at D2S1371; p = 0.00053). The gene encoding anion exchanger 3 (SLC4A3) has been mapped to this candidate region. SLC4A3 is prominently expressed in neurons and performs an electroneutral exchange of chloride and bicarbonate. To study the potential role of SLC4A3 in epileptogenesis, we performed linkage disequilibrium mapping in the region of 320 kb, harboring the SLC4A3 gene. Therefore, four SNPs (C_2915503, rs2305055, rs635311, and rs2891833) and one STR were genotyped in 415 German IGE patients and 275 healthy population controls. We found suggestive evidence for allelic associations of IGE with two adjacent SLC4A3 polymorphisms (rs2305055: p = 0.027; rs635311: p = 0.052). Haplotype analysis showed a significant association of both SLC4A3 polymorphisms with IGE (p = 0.015). Our results suggest that variation within the SLC4A3 gene confers a common but small susceptibility effect (OR » 2.2) to the aetiology of IGE syndromes. Considering that rs635311 encodes the amino acid substitution A867D, which is located in an extracellular loop near to a N-linked glycosylation site preceeding transmembrane segment M8, it is possible that the associated D867 allele itself mediates an epileptogenic effect.

P0798. Studies on the brain-expressed transcripts affected by a chromosome rearrangement in a female patient with severe brain malformations

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We have investigated the chromosome abnormalities in a female patient exhibiting a severe cognitive disability associated with complete agenesis of the corpus callosum and other gross anatomical brain malformations. The patient carries a balanced de novo translocation t(2;14)(p22;q13). Fluorescence in situ hybridisation led to the identification of breakpoint-spanning genomic clones on both chromosomes 2 and 14, and sequence analysis of the relevant regions indicated that one or more uncharacterised genes may be affected by the breakpoints. In view of the severe brain phenotype, the breakpoint on chromosome 14, which lies in a region harbouring genes and ESTs derived predominantly from brain tissue, became the focus of current studies. Fine-mapping by Southern hybridisation confirmed that the breakpoint lies approximately 6 kb from the 3' end of the well-characterised forkhead transcription factor FOXG1B, which is essential for normal development of the telencephalon in mice (Xuan et al. 1995). Human FOXG1B is an intronless gene with predominantly brain-restricted expression. Interestingly, our analysis of FOXG1B transcripts in foetal brain suggests the presence of several novel splice variants, the sequences of which are as yet undetermined, but may be affected by the breakpoint. Perhaps of even greater relevance is the fact that the chromosome 14 breakpoint interrupts the sequence of a novel, hitherto uncharacterised transcript. Northern blot analysis indicated that this transcript is approximately 8kb, and that it is expressed exclusively in foetal brain. Current studies aim to further characterise this transcript, and to determine its relationship to both FOXG1B and the patient phenotype.

P0799. Genetic instability of normal Spinocerebellar Ataxia type 8 (SCA8) alleles

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SCA8 is a dominant ataxia, associated with other neurological signs, caused by an untranslated CTG repeat expansion in the 3' end of the *SCA8* gene. Its transcript is an endogenous antisense RNA, overlapping the *Kelch-like 1* (*KLHL1*) gene, with unknown function. The CTG repeat is preceded by a polymorphic, stable $(CTA)_n$, in a configuration $(CTA)_{3-29}$-$(CTG)_n$, and is highly unstable. Large alleles are seldom found in the general population, which brought a lot of controversy to SCA8 expansion being the direct cause of disease. We previously reported a high germinal instability of the $(CTG)_n$ in both normal and expanded alleles. We have now 1) assessed the mutability region of this *locus*, by sequence analysis of the $(CTG)_n$ flanking region (~3460 bp), and 2) performed haplotype analysis with seven SNPs in five SCA8 and twenty control families. A high number of variations, including base substitutions, insertions and deletions were found within the examined region, but most were non-recurrent *de novo* variations; no significant difference as to the type or number of variations existed when SCA8 and control families were compared. Instability of normal alleles was observed in a maternal transmission to two sibs. Sequence analysis showed that also normal, not just expanded alleles, have interrupted CTA/CTG tracts. The only two haplotypes found in expanded chromosomes were also the most frequent in normal chromosomes from the control population. In conclusion, both expanded and normal alleles are fairly unstable during meiotic transmission, despite the conserved pattern of SCA8 haplotypes observed in the flanking region.

P0800. Factor IX gene mutations in Haemophilia B patients

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The clinical and molecular basis of hemophilia B is heterogeneous and the clinical severty is related to the levels of factor IX activity. The main purpose of this study was to perform molecular diagnosis of hemophilia B disease by DNA sequence analyses of the promoter, poly A and coding regions of the factor IX gene. Evaluation of the usage of technique in the diagnosis of factor IX gene mutations will light up further studies that aim to obtain mutation profile of the factor IX gene spesific to Turkish population.

By this aim, blood samples from thirteen clinically diagnosed hemophilia B patients were analysed by the automatic capiller gel electrophoresis tecnique. At least one mutation were identified in the patients, expect one case with mild hemophilia B of 11 identified molecular abnormalities, two were new mutations according to "Hemophilia B mutation bank" data of the recognised mutations, 33% were localised at the exon 8 of the factor IX gene. The frequency of mutations was higher in the CpG rich regions of the gene and missens mutations was regarded as the most common type of mutations causing severe hemophilia B and this conclusion was in accordance with the literature.

In conclusion, we suggested that capiller DNA sequencing is a fast and efficient approach to identify mutations in the factor IX gene and the results of this study will light up further studies in obtaining the factor IX gene mutation profile of the Turkish population.

P0801. Application of DHPLC for *FBN1* mutation detection: identification of 58 novel mutations

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Mutations in the human *FBN1* gene cause the Marfan syndrome (MFS), an autosomal dominant connective tissue disorder. Knowledge about *FBN1* mutations is important for early diagnosis, management, and genetic counseling of MFS. However, mutation detection in *FBN1* is a challenge because the gene is very large in size (~235 kb) and the ~600 mutations detected so far are scattered over all 65 exons, being largely unique to each affected family. While direct sequencing may be the most efficient way to identify a mutation in small genes, screening methods appear to be advisable for large genes such as *FBN1*. We have previously evaluated the sensitivity of DHPLC for *FBN1* mutation detection (89-99%, P = 0.05) and outlined a possible strategy for screening of point mutations and small insertions/deletions in *FBN1*.
We have applied this strategy to screen 94 unrelated patients with MFS or Marfan-like phenotype. We have thereby detected 76 *FBN1* mutations, 58 of which were novel and 3 recurred in unrelated patients. The mutations comprised 41 missense, 14 nonsense, and 12 splice site substitutions as well as 7 deletions, 1 insertion, and 1 insertion-deletion. We have also analyzed the presence of a *FBN1* mutation in a total of 139 members of 34 families by direct sequencing and identified the respective family specific mutation in a total of 75 relatives. Since DHPLC is less expensive than sequencing, the costs for mutation detection by using a combination of DHPLC and sequencing are lower than for that by sequencing alone.

P0802. Functional analysis of SLC7A7 mutations in renal tubular cells from patients affected by lysinuric protein intolerance.

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Lysinuric protein intolerance (LPI) is an autosomal recessive defect of cationic amino acids (CAA) transport at the basolateral membrane of epithelial cells in the intestine and kidney, caused by mutations of the SLC7A7 gene. We present results of a functional study of SLC7A7 mutations in the most suitable cellular system, i.e. renal tubular cells. Two independent patients, showing failure to thrive and severe tubular nephropathy, were found to be homozygous for the W242X mutation (patient 1) and homozygous for the R333M mutation (patient 2), respectively. This latter mutation, an apparently missense mutation, generates, indeed, a heterogeneous population of mutant transcripts. Both mutations were studied in renal tubular cells isolated from urine of the patients according to Inoue et coll. (Clin. Nephrol. 53:90-98, 2000). The origin of renal tubular cells was defined by appropriate immunohistochemical stainings. By filter uptake experiments, the arginine transport resulted strongly defective at the basolateral membrane. The use of renal tubular cells, isolated from urine samples, represents the best model to study the effect of a mutation in LPI and cognate diseases. This cellular model might also represent the most suitable system to test the efficacy of experimental therapeutic approaches.

P0803. Preparation of certified reference materials for hereditary haemochromatosis

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The use of appropriate Reference Materials (RMs) to validate test equipment or testing methods is an important part of any analytical testing system. Certified reference materials (CRMs) are RMs whose characteristics have been fully documented and validated. Currently, no CRMs are available for genetic testing. The CRMGEN project is a 14-centre collaboration funded by the European Commission's Measurement and Testing program*. We are developing reference measurement systems and producing CRMs for molecular genetic tests. Prototype RMs are being developed for a wide range of tests. These prototype RMs, developed in one of 4 genetics centres, are validated in 7 other centres before extensive field trials. The knowledge gained in this process is used to develop guidelines for the production of CRMs for any genetic test. Special emphasis is given to the commutability of the candidate RMs, i.e. their ability to perform under a wide range of test protocols and experimental set-up conditions.
PCR-based RMs were developed for hereditary haemochromatosis (HH) at the Dublin laboratory. These prototype RMs have reached an advanced stage of testing with the consortium having recently completed a field trial in conjunction with the European Molecular Genetics Quality Network. In addition, homogeneity and stability studies are currently being carried out at the JRC-IRMM, where a model system for certification of DNA-based RMs was established and described according to ISO guide 34 and the BCR guidelines. Different approaches for stabilisation of the prototype RMs are being investigated with respect to carrier DNAs and storage conditions.
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P0804. Mutation spectrum in the GFAP gene and clinical characteristics in patients with Alexander disease

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Alexander disease is a dominantly inherited disorder of cerebral white matter due to mutations in the glial fibrillary acidic protein gene (GFAP). The most common infantile form presents as mental retardation and megalencephalic leukodystrophy with onset in the first 1-2 years of life. Occurrence is usually sporadic caused by de novo mutations. Affected infants show delay of developmental milestones, macrocephaly, seizures, spasticity and rapid deterioration. Neuroradiological features include different white matter changes, enlarged ventricles, and basal ganglia abnormalities. Neuropathological examination reveals pathognomonic countless Rosenthal fibers, astrocytosis, and demyelination. Juvenile and adult forms of Alexander disease are characterized by a later onset and more slowly progressive course. It has been matter of a longstanding debate whether the juvenile and adult subtypes have the same etiology as infantile Alexander disease.
We have analyzed the GFAP gene in more than 30 patients with clinical and neuroradiological suspect of Alexander disease, most cases were diagnosed as infantile form. Missense mutations and rare sequence variants were detected in approx. 50% of cases. There was a clear clustering of mutations at the mutation hot spots in exon 1 and exon 4, affecting arginine 79 and arginine 239. However, also pathogenic mutations affecting non-arginine residues in other exons were identified, demonstrating the benefit of sequence analysis in all coding exons, in particular in cases with a clinical course characterized as juvenile or adult Alexander disease.

P0805. Further evidence for heterozygote advantage of *GJB2* deafness mutations: a link with cell survival

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Gap junctions composed of connexins (Cx) are intercellular channels that provide a mechanism of synchronised cellular response facilitating metabolic and electronic functions of the cell. Mutations in the *GJB2* gene that encodes Cx26 are the major cause of autosomal recessive non-syndromic hearing loss (NSHL). The high carrier frequency of these *GJB2* mutations in many ethnic groups suggests there may be a heterozygous advantage. A previous study has shown a link with the skin, specifically a thicker epidermis in heterozygotes and homozygotes for the R143W *GJB2* allele.
Here, we describe *in vitro* analysis of deafness-associated missense *GJB2* mutations that provide further evidence of a physiological mechanism that could provide *GJB2*- phenotypic advantage *in vivo*. Immortalized keratinocytes and other cell lines were transfected with various mutant and wildtype Cx26-EGFP constructs. 48 hours post transfection, unfixed cells were FACS analysed for EGFP expressing cells and also propidium iodide, the latter as an indicator of cell death. All cells transfected with the NSHL associated *GJB2* mutations resulted in reduced keratinocyte cell death compared to wildtype

GJB2 and dramatically less than the skin disease-associated *GJB2* mutations.
Increased cell survival may hint towards a cellular mechanism supporting a putative selective epidermal advantage for hearing loss associated *GJB2* mutations in different populations. Reduced cell death may extend the keratinocyte terminal differentiation programme resulting in a slightly thicker epidermis leading to improved barrier function against infection. Current studies are focusing on bacterial invasion by promotion of signalling events through various different Cx hemichannels in keratinocytes.

P0806. CHILD-syndrome: A metabolic disease with impact on development

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CHILD syndrome (*C*ongenital *H*emidysplasia with *I*chthyosiform *N*evus and *L*imb *D*efects, MIM 308050), is 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. Recently, we were able to demonstrate that this trait is caused by mutations in in the gene *NSDHL* (NAD(P)H steroid dehydrogenase-like protein) encoding a 3ß-hydroxy-steroid dehydrogenase functioning in the cholesterol biosynthetic pathway. *NSDHL* maps to Xq28. Mutational analysis in 30 familial and sporadic cases shows that the phenotype is 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. Missense mutations exchanging conserved amino acids outside the predicted functional domains (co-factor binding site, catalytically active site, transmembrane helix) identify positions of potential functional importance. Two human alleles of *NSDHL* (A105V and G205S), which have been observed in four and two unrelated females, respectively, were investigated for complementation in the *erg26*^{ts} yeast strain, which is mutated in the orthologous gene, *ERG26*. Two murine X-linked dominant male-lethal traits, bare patches (*Bpa*) and striated (*Str*) had previously been associated with mutations in *Nsdhl*. Remarkably, these mutants do not show a lateralization of the ichthyotic nevus. The developmental defects associated with lack of NSDHL suggest an impact on the hedgehog signaling pathway during development. Immunohistochemical analysis was applied to study this effect on determination of left-right asymmetry in *Bpa* mice during early development.

P0807. A New Mutation in the β -Globin Gene (IVS I-7 A→ T) Discovered in two Italian Families.

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Here we describe a novel mutation at nucleotide 7 of the first intron of the β -globin gene. The new mutation was found in four members of two different Italian families: two carriers (mother and her child) were identified on the first family while on the second one the A->T substitution at position IVS I -7 was found in a carrier woman and in a child with Thalassemia Intermedia phenotype. DNA study in Thalassemia Intermedia propositus showed a genetic compound characterized by the presence of the IVS-1 nt 7 A->T and the CODON 39 C->T mutations. Haematological and haemoglobin data were obtained with an automated cell counter, Hb A2 and HbF levels were quantitaded by HPLC analysis and globin chain synthesis was performed by the standard methods. Second step of investigation (molecular analysis) was performed by direct DNA sequencing.
To confirm the results ASO probes were synthesized for dot-blot hybridazation.
The new mutation can be considered a rare nucleotide defect since it was not identified among the uncharacterized β -thalassemic chromosomes from Mediterranean area.
In all subject whom the mutation is present as carrier state the haematological data exhibit a very mild microcytosis (MCV 79.9-88.2 fl, MCH 26.4-30.2 g/dl), an α /nonα ratio of 1.24-1.30 and a light increase of Hb A2 level (3.5-3.7%). These data can be superimposed with the IVS-1 nt 6 T->C haematological heterozygote phenotype. The COD 39/IVS 1-7 genetic compound seems to be associated with a mild non transfusional Thalassemia Intermedia phenotype.

P0808. Mutation screening of SLC26A4 by D-HPLC analysis: new mutations and phenotypic correlations in Pendred syndrome and DFNB4

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Recessive mutations of SLC26A4 (PDS) gene are a common cause of Pendred syndrome and non-syndromic deafness (DFNB4 type). Pendred syndrome is caracterised by sensorineural deafness, goitre and temporal bone abnormalities including dilated vestibular acqueduc (DVA) and Mondini dysplasia. DFNB4 is distinct from Pendred syndrome by absence of thyreomegaly. We performed by means of denaturing high performance liquid chromatography (D-HPLC) and DNA sequencing, molecular analysis of the SLC26A4 gene in a panel of 61 unrelated patients with phenotype compatible with Pendred syndrome or non-syndromic recessive type deafness. To date, we have characterized 12 deafness-causing alleles of SLC26A4. A patient with a typical Pendred syndrome was homozygote for the recurrent IVS8+1G>A splice mutation. Of patients with Mondini dysplasia or/and DVA but without thyreomegaly, three were compound heterozygotes and four carried only one disease-causing mutation. In conclusion, we show that D-HPLC is an efficient and cost effective methodology for the rapid screening of mutations in the 21 exons of the SLC26A4 gene. We report detailed clinical data on screened patients with confirmed DFNB4 to contribute in elucidating genotype-phenotype correlations.

P0809. A novel metal-binding protein in human mitochondria is involved in the biogenesis of the small Tim proteins

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The human small Tim proteins are soluble components of the mitochondrial intermembrane space (IMS), which are involved in the import and insertion of hydrophobic precursor proteins into the inner membrane. They belong to a family of evolutionary conserved proteins characterised by a common Cys4-zinc binding motif.
Little is known about the biogenesis of the small Tim proteins and how they are transported into the IMS. It has been suggested that binding of zinc via the Cys4 motif in the IMS might provide the driving force for the import of the small Tim proteins across the outer membrane.
We identified a novel mitochondrial protein of the IMS which might function as a metal delivery factor during biogenesis of the small Tim proteins. This protein also contains a metal binding site and is able to bind divalent cations such as Zn2+ and Cu2+. We used the human small Tim protein DDP1 (deafness dystonia peptide 1) as a substrate to analyse the function of this component in more detail. We could show that the import efficiency of DDP1 and its steady state protein level in the IMS is significantly increased in the presence of this component. We therefore suggest that the new metal-binding protein is involved in the biogenesis of the small Tim proteins by stabilising this proteins the IMS.

P0810. High frequency of heterozygous classical CYP21 mutations in women with nonclassical congenital adrenal hyperplasia (CAH)

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Introduction: Deficiency of 21-hydroxylase (21-OHD) is an autosomal recessive disease, caused by mutations in the 21-hydroxylase gene (CYP21). It is the most frequent CAH, characterized by hirsutism and prenatal virilization in females, accounting for more than 90% of CAH cases. The syndrome is divided into three groups according to severity of phenotype: classical forms (SW, salt-wasting; SV, simple

virilizing) and nonclassical (NC).
Patients: Examination for mutations in the CYP21 gene has been carried out in 67 unrelated female patients with suspected nonclassical 21-OHD, due to phenotype (hirsutism, acne, menstrual irregularities) and elevated base line and ACTH-stimulated levels of 17- hydroxyprogesterone (17OHP). These women were referred to our center with the diagnosis of primary or secondary infertility.
Results: Of those 67 patients evaluated, all patients carry at least one CYP21 mutation. 57% of patients carry one classical mutation (IVS2-13 A/C->G, deletion of 8nt, Q318X, R356W) or in combination with one of the nonclassical mutations (P30L, V281L, P453S). In two patients we identified novel mutations.
Conclusion: CYP21 mutations causing classical 21-OHD occurred with high frequency in women suspected for nonclassical CAH according to medical history, clinical symptoms and biochemical investigations.
Molecular analysis of CAH due to CYP21 mutations is one important step in diagnosis, counseling and treatment of women with signs of adrenal hyperandrogenism to prevent genital abnormalities in affected newborn girls.
Genetic counseling was recommended for all patients and partners. Prenatal therapy -in utero- using dexamethasone was advised in pregnancies at risk.

P0811. Genomic rearrangements at the IGHMBP2 gene locus in patients with SMARD1.

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Autosomal recessive spinal muscular atrophy with respiratory distress type 1 (SMARD1) is caused by mutations in the immunoglobulin µ-binding protein 2 gene (IGHMBP2). Patients affected by the infantile form of SMARD1 present with early onset respiratory distress within the first 13 months of life. So far, neither patients with juvenile onset nor with larger deletions/ rearrangements in IGHMBP2 have been reported. In this study we investigated one patient with infantile (4 months) and another with juvenile (4.3 years) onset of respiratory distress. Direct sequencing of all exons and flanking intron sequences in both patients had revealed a mutation on only one allele.
In both patients we identified genomic rearrangements on the other allele of IGHMBP2 by means of Southern blotting. Putative breakpoints were confirmed by PCR on genomic and cDNA.
The patient with juvenile onset had an Alu/ Alu mediated rearrangement, which resulted in a loss of ~18.5 kb. On mRNA level this caused an in-frame deletion of exons 3-7. The patient with infantile onset had a complex rearrangement with two deletions and an inversion between intron 10 and 14. This rearrangement led to a frameshift on mRNA level.
Our results show that SMARD1 can be caused by genomic rearrangements at the IGHMBP2 gene locus. This might be missed by mere sequence analysis. Additionally, we demonstrated that juvenile SMARD1 could be caused by mutations in the IGHMBP2 gene. The complex nature of the genomic rearrangement in patient 2 with infantile SMARD1 is discussed, and a deletion mechanism is proposed.

P0812. Molecular characterization of *WFS1* in five Danish patients with Wolfram syndrome

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Wolfram syndrome is a rare autosomal recessive disorder, caused by mutations in the *WFS1* gene on 4p16.1. The condition is also called DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness), and was first described in 1938. After cloning of the *WFS1* gene (1998), more than 161 disease mutations are known in the gene, 119 of which are associated with Wolfram syndrome, 19 in connection with low-tone sensorineural hearing impairment, and 23 in relation to diabetes mellitus or psychiatric disorders (http://www.khri.med.umich.edu/research/).
Five unrelated Danish patients with typical clinical WS presentation were included in sequencing of the entire gene and intron border regions. We identified six different *WFS1* mutations, of which four had not been publsihed before.
Patient 1 and 2 were homozygous for the mutations, F883fsX953 respectively delV415. Both probands had consanquineous parents.
Patient 3 was a compound heterozygote for the mutations, A133T and L543R. The identified mutations segregated in accordance with autosomal recessive inheritance, as expected for these three patients.
Patient 4 and 5 were shown to be heterozygous for the mutations, Q194X respectively H313Y. One parent to patient 4 carried the expected mutation, whereas in patient 5 the mutation was not found in any of the parents and therefore a new mutation.
In summery, our study contributes to the molecular understanding of this rare disorder and extends the spectrum of mutations to be associated with this syndrome in order for establishing a specific diagnosis, providing genetic counselling and the possibillity for prenatal diagnosis, whatever wanted by the families.

P0813. Gene dosage analysis of the *MECP2* gene detects hot spots deletions in patients with Rett syndrome and duplications in males with complicated mental retardation.

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MECP2 mutations are responsible for Rett syndrome (RTT). Approximately a quarter of classic RTT cases, however, do not have an identifiable mutation. We hypothesised that larger deletions arising from a deletion prone region (DPR) occur commonly and are not being routinely detected by the current PCR-mediated strategies. We developed and applied a quantitative PCR strategy (qPCR) to samples referred for diagnostic assessment from 140 patients among whom RTT was strongly suspected and from a second selected group of 31 girls with classical RTT. The investigation was restricted firstly to exon 3 and exon 4 and adjacent region of the MECP2 gene. We identified 10 large deletions (7.1%) within the first group and 5 deletions in the second group (16.1%). We found a majority of breakpoints in Alu repeats and in the DPR region that contains a perfect chi sequence, known to be recombinogenic in E. coli. We propose that the chi sequence and Alu repeats are potent recombinogenic factors. This hypothesis has been validated in an in vitro-model supporting the interaction between the chi sequence and Alu repeats. Furthermore, we have extended the qPCR to the complete *MECP2* gene including the 5' UTR and 3' UTR regions and tested 49 females and 71 males. Ongoing experiments are suggesting the existence of a possible second hot spot for deletions. Surprisingly, in two males the qPCR indicates the presence of a duplication of the *MECP2* gene and adjacent regions. Further experiments will focus on the characterization of the deletions and duplications.

P0814. Prevalence and Spectrum of Sarcomeric Gene Mutations in Hypertrophic Cardiomyopathy in an unselected Patient cohort

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Hypertrophic cardiomyopathy (HCM) is an autosomal dominat disease, characterized by myocardial hypertrophy and myofibrillar disarray. Clinical manifestation range from asymptomatic courses to chronic progressive heart failure and/or sudden cardiac death. HCM exhibits substantial inter- and intragenic heterogenity. More than 200 mutations have been identified within 11 genes, ten of them encoding for cardiac sarcomeric proteines. Screening of HCM patients for mutations in these genes provides important diagnostic and prognostic information. For HCM diagnostic, we developed an mixed strategy with DHPLC analysis of all exons of the TNNT2 gene and direct sequencing of MYH7 (exon 3-24) and MYBPC3.
For DHPLC screening of TNNT2 all amplicons were adjusted to one common PCR profile and fragments were run along with controls in one DHPLC run, thus complete analysis of TNNT2 for 6 Patients can be performed in one week. In 92 unrelated index patients with

a completed analysis of TNNT2, we found 3 patients (3.3%) with a mutation, all displaying the same mutation (R92W).
62 of these patients were further screened for mutations in MYH7 and MYBPC3. In total, 21 mutations were found. 10 mutations were found in MYBPC3, 9 in MYH7 and only 2 patients had TNNT2 mutations.
Previous reports estimated the frequency of TNNT2 mutations in HCM patients as 15-20%. In our experience and according to recently published data, this frequency seems to much lower in the range of 2-5%.

P0815. A novel frameshift mutation in the SRY gene detected in a family presenting complete gonadal dysgenesis (Swyer syndrome)

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Mutations in the testis-determining gene SRY have been shown to result in XY sex reversal with pure gonadal dysgenesis. Most of the mutations that have been described fall into a DNA binding domain termed HMG box. In addition, there are several cases in the literature of familial gonadal dysgenesis where the SRY mutation is shared by several of the siblings within a family. We present here a novel familial nonsense mutation that is shared by two XY sisters but not by their normal brother. The two sisters (28 and 31 years of age) show complete sex reversal with pure gonadal dysgenesis, and both have developed bilateral gonadoblastoma and dysgerminoma that arose from their streak gonads. The mutation consists of an insertion of a cytosine at nucleotide position 226, in between position 3 of codon 76 and position 1 of codon 77 within the HMG box. This insertion results in a change of the amino acid sequence from this point on and a premature stop codon 26 amino acids downstream. Presumably, the mutation results in a truncated protein with most of the HMG box missing. Interestingly, although the two sisters present the same mutation we have not detected it in genomic DNA extracted from peripheral blood of their normal father. This suggests a low frequency mosaicism absent in blood but present in the father's germinal line.

P0816. Alanine-expansions in Hoxd13, causative for Synpolydactyly, lead to protein accumulations in the cytoplasm

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Hox genes are master regulators of development that control patterning and morphogenesis. They play a major role during limb development as illustrated by gene inactivation experiments in mice and mutation analysis in human malformations.
We have investigated the human synpolydactyly (SPD). The phenotype is caused by the expansion of a polyalanine encoding repeat in the 5' region of the *Hoxd13* gene. Different alanine-expansions from +7 to +14 alanines are found and the penetrance and severity of the phenotype increases with the increase of the expansion. The phenotype consists of central polydactyly, syndactyly and brachydactyly.
To investigate wether the alanine-expansion has an effect on the distribution of the protein in the cell, we transiently transfected COS-1 cells with an expression vector containing tagged Hoxd13wt and mutant proteins, with different expansions, from +7 to +21 alanines. The wt protein is always localized in the nucleus. The mutant proteins show localization in the nucleus as well as accumulation in the cytoplasm. Interestingly, the number of cells with protein accumulations increased with the increasing number of alanines. This could be an explanation for the increase of penetrance and severity of the phenotype in SPD patients.
Our results indicate that alanine expansions in Hoxd13 results in misfolding of the protein which cummulates in the cytoplasm, thus preventing the protein from entering the nucleus.

P0817. Demonstration of increased SHP-2 phosphatase activity in lymphocytes of Noonan-Patients and recombinat SHP-2 protein and mutation dependant downregulation of STAT-1 pathway

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Mutations in the PTPN11-gene, coding for the non-receptor tyrosine phosphataseSHP-2, are causative for Noonan-Syndrome I (NSI) and for LEOPARD-Syndrom (LS).
Based of protein conformation modelling and theoretical considerations it was postulated that Noonan-typical mutation exert a gain of function by inducing increased or deregulated SHP-2 phosphatase activity.
We investigated the biochemical effect of several recurrent and of novel PTPN11 mutations and demonstrate an approximately 2-fold increase of SHP-2 phosphatse activity both in EBV transformed lymphocytes of NS-patients and in recombinant SHP-2 protein (wt vs. mutated).
Because SHP-2 is known to dephosphorylize phosphorylated STAT1-protein, a major signal transduction factor, we sought to demonstrate the biological effect of deregulated SHP-2 phosphatase activity on intracellular signaling pathway. SOCS-1 is an acute phase protein which acts as a negative feedback regulator of IL-6 and Ifnγ induced stimuli and is mainly regulated via the JAK/STAT pathway, namely STAT1. In a promoter/reporter-assay we showed that cotransfection of SOCS-1-promoter/luciferase-plasmid together with mutant SHP-2 plasmid results in a downregulation SOCS-1 promoter actvity.
Our data for the first time provide experimental evidence that PTPN11-mutation act via a gain of function mechanism and demonstrate a biological effect on intracellular signalling.

P0818. Resequencing the whole CFTR gene as a new microarray-based diagnostic tool

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Cystic fibrosis (CF) is the most common genetic disease among Caucasians. The CF gene (27 exons) encodes for a protein that acts as an epithelian membrane channel. Making an accurate, early diagnosis is essential to the management of the disease. The recent development of a genotypic CFTR mutation screening has greatly improved diagnostic accuracy, but the direct sequencing of the whole CFTR gene is very expensive and time-consuming.
Here we report the first microarray-based resequencing analysis of the complete CFTR gene. For this purpose, we used the oligonucleotide-microarray technology and designed a CustomSeq-Array for the whole CFTR gene sequence. The hybridisation analysis is a promising new technology which potentially allows rapid and cost-effective screen for all possible mutations and sequence variations in genomic DNA.
Twenty-eight unrelated CF patients were analysed. All 27 exons of each sample were amplified by PCR using specific primers, pooled, labelled, fragmented, and hybridised to the CFTR-CustomSeq array. In addition all samples were confirmed by conventional sequencing procedure.
All analysed CFTR mutations could be detected by the new diagnostic system. The CFTR-array provides base calls at more than 99.99 accuracy compared to capillary sequencing. Replicate experiments demonstrated a reproducibility of more than 99.99%.
Our experiments with a microarray-based sequencing of the CFTR gene demonstrates a new efficient and cost effective method for analyzing large amounts of sequence and opened a new dimension of genetic diagnostics.

P0819. Mutations in SLC6A8 (Creatine Transporter) Are Responsible For About 1% of Mental Retardation in Males

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Mutations in the creatine transporter gene, SLC6A8, located in Xq28, have been found in families with X-linked mental retardation (XLMR) as well as some males with MR. In order to estimate the frequency of mutations in SLC6A8 in the MR population, a screening of 501 males with MR of unknown cause was undertaken. All 13 exons of SLC6A8 were sequenced using genomic DNA. Seven causative mutations were identified: 4 missense, 2 deletions and 1 splice site alteration. For the missense mutations and the splice site change, testing of 500 X chromosomes from normal males failed to detect any of the changes. Common clinical findings in the patients with missense or deletion mutations were the presence of seizures and behavior problems. Our findings of 7 mutations in 501 samples would indicate about 1.4% of males with MR might have a SLC6A8 mutation. Thus, DNA sequence analysis might be warranted in any male with MR of unknown cause. Alternatively, since mutations in the gene are associated with an increased creatine/creatinine ratio in urine, a urine screen in this same population may prove valuable.

P0820. Confirmation of complex dystrophin gene mutations applying the MPLA-method

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Deletions/ insertions of whole exons in carrier women and insertional mutations in male patients are difficult to detect in the large dystrophin gene using routine screening methods, e.g. multiplex PCR, Southern blot, FISH analysis and RT-PCR fragment analysis from muscle mRNA. Now a quantitative multiplex ligation dependent probe amplification assay (MPLA, MRC Holland, Amsterdam) for the dystrophin gene is available. Two mixes of 39 or 40 exon-specific probe pairs, each, with universal primer sequences were applied in two reactions, specifically ligated and multiplex amplified with fluorescently labelled universal primers. The multiple fragments were visualized on a DNA sequencer (Beckman CEQ 2000) and validated by internal controls.
Two difficult diagnostical cases could be solved. A symptomatic female carrier was suspicious to have an out of frame duplication of exons 6 and 7 of the dystrophin gene. RT-PCR of mRNA isolated from muscle tissue gave only a normal sized fragment (apparently nonsense mRNA decay of mutated RNA). The MPLA analysis confirmed an isolated exon 7 duplication. In a male patient with DMD phenotype and mental retardation we detected an in frame deletion of exons 45-47 by multiplex PCR. But Western blot analysis of muscle tissue showed no dystrophin protein. Using RT-PCR mRNA analysis an aberrant fragment of this region could be directly sequenced. A tandem duplication of exons 43/44 followed by a deletion of exons 45-47 was suggested. The complicated mutational pattern was likewise confirmed by MPLA method.

P0821. Molecular analysis of the Alpha-Sarcoglycan, Gamma-Sarcoglycan and Caveolin-3 genes in a serie of patients with Limb Girdle Muscular Dystrophy.

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Limb Girdle Muscular Dystrophies (LGMD) constitue an heterogenous group of autosomal dominant (LGMD1) or recessive (LGMD2) muscular disorders.
Among LGMD2, sarcoglycanopathies (LGMD2C-F) are caused by mutations in the genes encoding Gamma-,Alpha-,Beta- and Delta-Sarcoglycans respectively.
Among LGMD1, LGMD1C is caused by mutations in the gene encoding Caveolin-3.
Our laboratory in Marseille has developed the molecular testing for mutations in the Alpha- and Gamma-Sarcoglycan genes (SSCP screening followed by direct sequencing of electrophoretic variants) and in the gene encoding Caveolin-3 (direct sequencing) on a routine basis.
In a serie of 11 patients, we identified mutations in the Alpha- or Gamma-Sarcoglycan genes for 6 individuals having a caracteristic LGMD phenotype according to established criteria, and with transmission compatible with an autosomal recessive mode of inheritance.
In our serie of 20 patients suffering from LGMD of likely autosomal dominant mode of inheritance, we identified only one mutation in the gene encoding Caveoline-3. The patient, 71 years old at diagnosis, seemingly is the first reported case of late-onset LGMD1C. The identified mutation (R27Q) has been reported before in patients suffering from rippling muscle disease, isolated hyperCKaemia or distal myopathy (in one case). Our case report illustrates the implication of this mutation in a fourth phenotype, and the high phenotypic variablility associated with mutations in the gene encoding Caveolin-3, even for the same mutation. Further studies should lead to the identification of genetic or non-genetic factors modulating the phenotype associated with these mutations.

P0822. Defects in O-glycosylation associated with muscular dystrophy and lissencephaly

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Defects in O-glycosylation have recently been identified as the cause of a spectrum of autosomal recessive congenital disorders, affecting both muscle function and the central nervous system. Mutations in the gene for protein O-mannose-beta-1,2-N-acetylglucosaminyltransferase (*POMGnT1*, OMIM 606822) cause Muscle-Eye-Brain Disease (MEB, OMIM 253280) and in addition have been reported in patients with severe forms of Fukuyama-Congenital-Muscular-Dystrophy (FCMD, OMIM 253800) or mild forms of Walker-Warburg-Syndrome (WWS, OMIM 236670). In other probands with WWS mutations in the gene for protein O-mannosyltransferase (*POMT1*, OMIM 607423) have been identified.
In all these different syndromes the clinical features include congenital muscular dystrophy, brain malformations and ocular abnormalities. The MRIs or CT scans reveal cobblestone lissencephaly (lissencephaly type II) with pachygyria or polymicrogyria and sometimes also hydrocephalus. The cerebellum can present only with cysts or can be severely hypoplastic. There is usually an atrophy of the brain stem. The eyes can show anterior chamber anomalies with colobomas, glaucoma, or cataracts, myopia and retinal dysplasia. The patients are often severely mentally retarded, present with severe hypotonia and weakness, and very often with epileptic seizures.
Here, we report the genetic analysis and clinical data of 3 probands with characteristic phenotypic features of WWS or MEB including muscular dystrophy and brain malformations, where we have found 3 novel mutations within the *POMGnT1* gene.
Mutation detection in those probands will not only support genetic counseling of the affected families but also provide new insights into the importance of O-glycosylation for the proper formation of cortex and cerebellum.

P0823. Molecular analysis of Pyruvate Kinase deficiency using dHPLC : a 3 year study

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Pyruvate Kinase deficiency (PKRdef)(OMIM : 266200) is one of the most frequent anomalies of the energetic pathway of erythrocytes. It induces a chronic anemia of variable severity from silent carrier to severe alpha thalassemia like disease. Research for molecular anomaly of PKLR gene (1q21) has shown that 80% of mutants are of private type located on the 11 exons, with only 3 frequent mutations : R486W, R510Q and E241X. Using dHPLC, we have screened the PKLR gene in 49 families affected by PKRdef. We founded a mutation in 65 from the 86 potentially affected alleles including

R486W (10 alleles), R510Q (7 alleles) and E241X (4 alleles). 25 new mutations were described. This result is consistent with the expected frequency of private and frequent mutations in PKRdef. In 34 from the 49 families, we found a genotype in agreement with the phenotype, only 1 allele in 7 families, 0 alleles in 8 families (overall sensitivity of 75%). Two hypotheses may explain such result : 1- the existence of other molecular pathology as deletion. We have designed a QMPSF test to search for deletion in PKLR gene. A deletion removing from exon 4 to 10 was found . 2- The PKLR gene may not be involved in the 8 last families. This hypothesis brings sensitivity up to 92% with is close to what is expected from dHPLC mutation screening. Since enzymatic measurement in erythrocytes is prone to artifact, molecular screening of the PKLR gene may be a part of the workup for the etiology of chronic anemia.

P0824. Increased copy number of DAZ genes in subfertile men

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Deletions of the AZFc (azoospermia factor c) region are the most common known cause of spermatogenic failure. Different genes map to AZFc but the strongest candidate is DAZ (deleted in azoospermia) gene family, which consists of four nearly identical gene copies. Recent studies indicate that a minority (8%) of patients with severe oligozoospermia have a DAZ1 and DAZ2 gene deletion that is mediated by homologous recombination between duplicons termed gr. The reciprocal products of these deletion events, partial AZFc duplications, have not been reported to date.
Therefore, our objectives in this study were: (i) to determine whether DAZ genes are prone to duplications, (ii) to determine whether DAZ gene duplications are associated with male infertility, (iii) to re-evaluate the importance of partial DAZ gene deletions in subfertile men.
Ninety patients with male idiopathic subfertility (44 azoospermics and 46 oligozoospermic) and forty-seven fertile men controls were analysed using real time PCR. The incidence of two DAZ gene copies was 21,7% (10/46) in oligozoopermic men, 9% (4/44) in azoospermic men and 6,4% (3/47) in control group. Six or eight DAZ gene copies were only found in oligozoospermic (6,5%; 3/46) and azoospermic (6,8%; 3/44) men. Increased number of DAZ gene copies could be a risk factor for spermatogenic failure. Partial DAZ gene deletions are common in oligozoospermic but not in azoospermic men. Real -time PCR is a valuable, simple and fast method for the determination of number of DAZ gene copies.

P0825. Comprehensive mutation analysis is not possible by direct sequencing alone

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Direct sequencing is often considered the “gold standard” for detection of unknown mutations. Many mutations, hoever, cannot be detected by direct sequencing.
The most common causes are:
- exclusive amplification of the wild type allele due to a deletion encompassing at least one of the PCR primers
- asymmetric PCR due to a polymorphism in one of the primer annealing sites
- somatic mosaicism
- splice errors due to mutations far away from the exons

Comprehensive detection of unknown mutations requires a combination of tests. Large deletions and duplications can be readily detected by quantitative methods, such as MLPA (multiplex ligation dependent probe amplification), testing all exons of a gene. Proper primer design, avoiding polymorphic sites, can solve the problem of asymmetric PCR in the sequence analysis of genomic DNA, but does not help in the detection of somatic mosaicism. Splice errors can be detected by sequencing of cDNA, but this may be insensitive due to loss of the mutant mRNA by instability or nonsense mediated decay. We have successfully used DHPLC (denaturing high performance liquid chromatography) as a pre-screening method for the detection of somatic mosaicism in genomic DNA as well as splice errors and nonsense mutations in cDNA. Some of these mutations in the genes *COL1A1, COL1A2, COL3A1* and *FBN1* could not be detected by direct sequencing alone, either due to the low level of mosaicism or because the causative mutation was located hundreds of bases away from the exons.

P0826. The spinocerebellar ataxia type 10 ATTCT repeat is present in Brazilian SCA families, but not in Portuguese subjects affected with ataxia

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Spinocerebellar ataxia type 10 (SCA10) is a rare autosomal dominant neurodegenerative disorder mainly characterized by gait and limb ataxia, and seizures. The disease-causing mutation is the unstable expansion of an ATTCT repeat, located on intron 9 of the *SCA10* gene: the normal range is 10 to 22 pentanucleotide repeats, while expanded alleles range from 800 to 4500 units. SCA10 was first found in 5 families of Mexican origin and, recently, Brazilian families were also described.
We have ascertained and studied 274 unrelated individuals, representing 82 families with autosomal dominant ataxia, 31 apparently recessive ataxia kindreds and 161 isolated cases; 234 were of Portuguese origin, while 40 were from Brazil. Clinical manifestations included progressive gait ataxia, associated with, seizures, mental retardation or tremor. A modified PCR-based method was used to detect the repeat expansion, followed by 6% polyacrylamide gel electrophoresis and hybridization with a ^{32}P labelled ATTCT probe.
We detected an ATTCT repeat expansion in one family (2 affected individuals) with autosomal dominant transmission and in an isolated case, all of Brazilian origin, and presenting with a progressive cerebellar ataxia without seizures. No mutation was found in any of the Portuguese subjects. In conclusion, our results did not show the presence of the SCA10 expansion in any of the Portuguese ataxia families studied, confirming that this is a rare form of hereditary ataxia, confined, until now, to the Mexican and Brazilian populations.

P0827. Molecular vs. clinical diagnosis in Bartter and Gitelman syndromes

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Gitelman (GS) and Bartter (BS) syndromes are a group of closely related hereditary tubulopathies, characterised by renal salt wasting, hypokalaemic metabolic alkalosis, normal blood pressure and hyperreninaemic hyperaldosteronism. Loss-of-function mutations have been identified in four genes involved in transepithelial transport of sodium chloride in the renal tubules: the SLC12A1 and SLC12A3 genes that encode the NKCC2 and the NCCT cotransporters, respectively; the CLCNKB and KCNJ1 genes encoding for the ClC-Kb chloride channel and the ROMK potassium channel, respectively. Ante/neonatal form of Bartter syndrome (type I and II) is caused by mutations in NKCC2 cotransporter or ROMK channel and patients present similar characteristic clinical and biochemical data. The ante/neonatal Bartter is easily differentiated from the other two forms; Gitelman and classic Bartter (type III) syndromes overlap phenotypically and it is an expensive and time consuming process to identify the underlying molecular defects of an increasingly number of patients. In the past years, the molecular genotyping of those patients carried out on the SLC12A3 and CLCNKB genes allowed some observations, principally based on the evaluation of the initial presentation and laboratory findings at the onset. The aim of this study was to elucidate, through a retrospective phenotype-genotype correlation, the parameters constituting the lowest common denominator for each syndrome. Consequently, a flow chart is

proposed where this more precise clinical classification is integrated with a molecular approach focusing those regions of the genes that are mostly involved in the disease-causative mutations in the Italian population.

P0828. MDR3 gene analysis in PFIC3/ICP patients and as a modifier gene of liver disease associated with Cystic Fibrosis

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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, and of Intrahepatic Cholestasis of Pregnancy with high serum gGT activity (ICP).
Cystic Fibrosis patients with liver disease show some features in common with patients affected by PFIC3: high gGT values, evidence of inflammation of the portal tracts, bile duct proliferation and fibrosis at liver biopsy, cholelitiasis.
Our study has two specific aims: - to identify MDR3 gene mutations in PFIC3 and ICP patients; - to determine whether CF patients heterozygous for a MDR3 mutations or for a specific MDR3 polymorphism may be at higher risk for development of liver disease.
We analyzed 9 patients with clinical findings of PFIC3 and identified three different mutations. Two new mutations: a heterozygous Arg149Stop mutation in one patient, a homozygous 2202insG mutation in the second patients, and heterozygous mutations in the consanguineous parents, were found. We also characterize one already known mutation Thr175Ala in the third patient.
We analyse also 30 CF patients with hepatic involvement and we characterise one known mutation (Thr175Ala), and 16 different nucleotide polymorphisms, but these results are not adequate to get statistical correlations.

P0829. Role of the putative pyrophosphate transporter ANK in skeletal homeostasis

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Missense mutations in ANKH were found to underlie the dominantly inherited disorder craniometaphyseal dysplasia (CMD), which is characterized by erlenmeyer-flask deformity of the distal femur and sclerosis of the long bones and the skull. In contrast, loss of function of the orthologous ANK in the mouse mutant ankylosis (ank) leads to joint destruction due to articular calcifications. We therefore rather assumed a gain of function effect of CMD mutations. Although ANK is a membrane protein that is thought to regulate the release of pyrophosphate (PPi) in various cell types, no significant changes could be observed in plasma PPi levels in CMD patients. Only subtle differences in subcellular distribution could be detected upon transient expression of mutant ANK compared to the wildtype protein. Hence, missorting or protein instability seems to be only of minor importance for the pathogenesis. Detection of ANK in murine bone tissue by in situ hybridization and RT-PCR clearly demonstrated expression in osteoblasts and osteoclasts. As an in vivo model for a loss of ANK function we investigated bone histology and bone density in the ank mouse mutant. These mice showed a reduction of bone density between of up to 30% before the onset of the characteristic ankylosis phenotype. This proves that ANK is able to directly influence bone density.

P0830. Mutations in the PQBP-1 Gene Cause Renpenning Syndrome and XLMR in Two Other Families with Microcephaly

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Renpenning et al (1962) decribed a family with X-linked mental retardation (XLMR) in males with microcephaly, short stature and small testes. The term “Renpenning syndrome” became a generic name for XLMR until the 1980’s. We had previously mapped the syndrome to Xp11.2-p11.4. Recently, mutations in the PQBP-1 gene were reported by Kalscheuer et al. (2003) in the Sutherland-Haan syndrome and 4 other families with microcephaly. Since the gene resided in the Renpenning linkage region, it was screened in the family as well as 16 other linked families. We found a truncating mutation in exon 5 (c.641insC) in the Renpenning family as well as 2 other truncating mutations in exon 4 in 2 XLMR families: c.459-462delAGAG and c.575-576delAG. The c.459-462delAGAG was reported previously while the other two are novel mutations. It is interesting that all mutations thus far identified in PQBP-1 are truncating mutations. However, some affect the DR/ER repeat (PRD) and two (c.641insC and c.575-576delAG) leave the PR-domain intact. It is not clear at this time whether the PR-domain in the latter two proteins still interacts with other proteins. Nonetheless, all families with PQBP-1 mutations have microcephaly and short stature. In a study in South Carolina of 4000 males with MR of unknown cause, 12% (486) had microcephaly, 9% (350) had short stature and 3% (128) had both. Based on these observations, it seemed appropriate to use these clinical measurements to select males with MR for PQBP-1 testing.

P0831. Variable phenotype in CADASIL -genetic background or environmental factors?

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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 dementia. The disease is caused by missense mutations or small deletions in the *NOTCH3* gene. At least 97 different disease-causing mutations have been published. The clinical course of the disease can be highly variable in different CADASIL families but also among individual patients in a same family. We describe identical twins, both having the clinical picture of CADASIL and diagnostically confirmed by skin biopsy, show significant differences in many clinical studies.
In Finland we have almost 100 CADASIL patients sharing the same ancestral *NOTCH3* mutation, R133C. We have studied clinical variation in this genetically uniform patient group. Studies include magnetic resonance imaging (MRI), positron emission tomography (PET) and neuropsychology.
We selected two groups of patients having the same mutation, R133C: 14 patients with early onset and 13 patients with late onset. We have tested 7 *NOTCH3* amino acid changing polymorphisms in both of these groups. None of the polymorphisms correlated with the phenotype and therefore do not affect the clinical variation.

P0832. Acid Sphingomyelinase: identification of nine novel mutations among Italian Niemann Pick type B patients and characterization of *in vivo* functional in-frame start codon

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Niemann Pick disease (NPD) is an autosomal recessive disorder due to the deficit of lysosomal acid sphingomyelinase, which results in intracellular accumulation of sphingomyelin. In the present work we studied 18 patients with NPD type B, including five individuals who presented an intermediate phenotype characterised by different levels of neurological involvement. We identified nine novel mutations including six single base changes (M1T, W32X, L103P, L225P, W244C, A281T) and three frameshift mutations (G34fsX76, P189fsX190 and P192fsX206).
The novel M1T mutation inactivates the first in-frame translation start site of the SMPD1 gene and in the homozygous status causes NPD type B indicating that *in vivo* translation of wild type SMPD1 initiates from the first in-frame ATG. Moreover, the new W32X introduces a premature stop codon before the second in-frame ATG (ATG33). As a consequence of either M1T or W32X, impaired translation from the first in-frame ATG results in a mild NPD-B phenotype instead of the severe phenotype expected for a complete deficiency of the enzyme, suggesting that when the first ATG is not functional, the ATG33 still produces a fairly functional sphingomyelinase.
Analysis of the patients'clinical and molecular data demonstrated that all five patients with the intermediate phenotype carried at least one severe mutation. No association between the onset of pulmonary symptoms and genotype was observed. Finally, the presence of W32X, the most frequent allele among Italian NPD type B population, and R600P as compound heterozygotes in association with severe mutations suggested a beneficial effect for both mutations.

P0833. Screening for ARX gene mutations is indicated for males with mental retardation associated with West syndrome and/or dystonia, or in XLMR families linked to Xp22.1

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The ARX gene (X-linked Aristaless Gene), localised in Xp22.1, has recently been implicated in both syndromic form of X linked mental retardation (associated with West syndrome and/or dystonia) and in non-specific form. A duplication of 24 bp in exon 2 (428-451dup(24)bp) was found to be a recurrent mutation. This duplication leads to an expansion of a polyalanine tract in the ARX protein.
The ARX gene was screened for mutations in five males with mental retardation associated with West syndrome and/or dystonia. The recurrent 24 bp mutation in the ARX gene was found in 2 patients. We also screened the ARX gene in a large family, in which we had previously mapped the mental retardation to a 23 cM region in Xp22.1, containing the ARX gene. We identified the ARX exon 2 recurrent mutation in this family. Recent studies seem to indicate that the contribution of mutations in ARX to isolated mental retardation in males is not that significant and therefore do not recommend an ARX mutation screening in these cases. On the other hand, our results show that this mutation screening is indicated both in case of mental retardation associated with West syndrome and/or dystonia, and in X linked mental retardation families mapped to the Xp22.1 region.

P0834. Characterization of the human carnitine-acylcarnitine translocase promoter

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The carnitine-acylcarnitine translocase (CACT) deficiency, one of the most severe of fatty-acid oxidation disorder, is characterized by hypoketotic hypoglycemia, hyperammonemia cardiac abnormalities and early death. Although considerable insight has been gained on the function of the carnitine-acylcarnitine translocase and its gene structure, surprisingly little information is available related to CACT transcriptional regulation. In this study, we have begun identifying and functional charactering the CACT regulatory region. Using the computer program to search human CACT promoter region, a putative functional promoter spanning a TATA box from -815 to -811 was recognized. To identify the minimal promoter sequences and potential positive and negative regulating regions, the series of fragments containing the CACT 5' UTR controlling the firefly luciferase reporter gene were cloned in the pGL3-Basic plasmid . The promoter-luciferase chimeric plasmids were transiently transfected into human hepartocellular carcinoma cells. The pRL-TK plasmid carrying a Renilla luciferase gene driven by the herpes simplex virus thymidine kinase promoter was co-transfected as the internal control. Four independent experiments showed that the highest relative activity localized to a region spanning -916 to -687 of the 5' flanking region. The loss of this 229 bp fragment resulted in significant loss of luciferase expression.The consistent results also suggested that the transcriptional repressors may bind between -686 to -416 bp. Identification of the transcription factor important for CACT gene expression will permit us to examine the phenotypic variability in CACT deficiency. In addition, in our five patients with CACT deficiency, the correlation of genotype to phenotype was also discussed.

P0835. Presence of elevated lactate, lactate/pyruvate ratio and acylcarnitine profile in patients with autism.

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Autism is defined behaviorally as a syndrome consisting of abnormal social skills development, sensorimotor deficits, and communication problems. The study of this disorder has intensified because incidence is as high as 6 in 1000 individuals. Although etiology is unknown, neuropsychiatry problems, lead poisoning, genetic and metabolic disorders have been implied in the pathophysiology of the disease. The clinical phenotype is divided according to the level of functioning of the individual ranging from autism as the most severe; pervasive developmental disorder; and Asperger syndrome. These patients are usually referred to Genetic clinics for evaluation.
65 patients with the diagnosis of autism or pervasive developmental disorder were evaluated. Thorough past medical history and physical examination was performed and family history was taken via pedigree. Laboratory evaluation included plasma amino acid levels, plasma lactate, pyruvate and ammonia levels, urine organic acids, plasma carnitine and acylcarnitine profiles and if dysmorphic features were identified blood chromosomes were also included. Out of the 65 patients; 49 returned with completed metabolic work up. In this group of 49 patients 18 had a diagnosis of autism and 31 of PDD, only one patient was female, average age was 5.4 years. Sixty percent of the patients had elevated lactate levels; 37% had decreased pyruvate levels; 60% had elevated lactate/pyruvate ratio and unspecific elevations in acylcarnitine profile. Even though these results are not conclusive this metabolic alterations point toward the possibility of an oxidative metabolism disorder or an intra-mitochondrial disorder to be involved in the expression of the autism phenotype.

P0836. Two new mutations in type 3 von Willebrand Disease: association of the mutation 7082-2A>G to very low expression of von Willebrand Factor gene.

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Type 3 von Willebrand disease (VWD) is a severe bleeding disorder, characterized by very low to undetectable levels of von Willebrand Factor (VWF) in plasma. VWF is expressed in platelets and endothelial cells. The heredity is recessive. This phenotype is caused by defects in VWF gene, which is about 178 Kb in length and contains 52 exons.

The segregation analysis was carried out with intragenic markers in nine members from one family. The haplotypes indicated the type 3 VWD patient received different alleles from his parents. Two exonic restriction analyses showed the patient was heterozygote in genomic DNA, while he showed homozygote patterns in the cDNA, obtained by inverse retrotranscriptase from platelet mRNA. This result demonstrated a null allele linked to father branche.
Mutation screening in coding regions and their boundaries exon/ intron of the VWF gene, realised by SSCP analyses and automatic sequencing, showed the patient carried the 449T>C (L150P) mutation in the exon 5, linked to mother allele, and the 7082-2A>G change affecting the splicing of the exon 42, associated to father allele.
All theses results allowed us to conclude the patient is a compound heterozygote, and his phenotype is caused by a missense mutation in the propeptide and a splicing mutation in the C1 domain of VWF close to the end of mature protein. These mutations have not been previously reported. It is the first time the mutation 7082-2A>G, located in the splice acceptor site, associated to a very low or null expression of VW gene, is described.

P0837. Mutational spectrum of *ENPP1* in 23 unrelated patients with infantile arterial calcification

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Background: Infantile arterial calcification (IAC) is characterized by calcification of the internal elastic lamina of large and medium-sized arteries and stenosis due to myointimal proliferation. Sometimes peri-articular calcification is also observed. Although survival to adulthood has been reported, most patients die within the first six months of life. Recently, we identified mutations of the *ENPP1* gene as the cause of this recessive genetic defect (OMIM 208000). *ENPP1* encodes ecto-nucleotide pyrophosphatase/ phosphodiesterase 1. This cell surface enzyme generates inorganic pyrophosphate (PP_i), a solute that regulates cell differentiation and serves as an essential physiologic inhibitor of calcification. Mutations were shown to dramatically reduce enzyme activity. **Methods and results:** We evaluated a total of 23 unrelated patients with typical IAC. All 25 exons including their flanking splice sites and the promoter region of *ENPP1* were amplified by PCR and sequenced bi-directionally from genomic DNA. We identified 22 different homozygous or compound heterozygous mutations in 18 of the 23 patients. The mutations, 3 nonsense, 14 missense, 1 single amino acid deletion, 3 frame shift, and 1 splice mutation, were scattered over the whole coding region. We identified 3 recurrent mutations (7 x P305T, 4 x R774C, 2 x N792S), consanguineous families not included. Mutation P305T seemed to be the result of a founder effect of British extraction while mutation R774C (CpG à TpG mutation) occurred repeatedly as revealed by haplotype analyses.
Conclusion: Mutation screening in IAC patients should start with *ENPP1* exons 8 and 23, harboring mutations P305T and R774C, respectively.

P0838. Subtelomere analysis in a cohort of individuals with mental retardation utilizing multiplex ligation-dependent probe amplification (MLPA)

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Chromosome alterations involving the subtelomeric regions have been found to be the causative genetic factor in 5-7% of individuals with mental retardation. The most common method for identifying these alterations has been fluorescent in situ hybridization (FISH) using a panel of probes on metaphase spreads. Unfortunately, doing cytogenetic FISH-based testing for subtelomere rearrangements is expensive and time-consuming. Therefore, alternative testing methodologies have been pursued in order to expedite laboratory turnaround times and to reduce costs. One such methodology is a PCR-based commercially available assay known as multiplex ligation-dependent probe amplification (MLPA). This methodology allows relatively large sample volumes to be run simultaneously, significantly reducing laboratory turnaround times. In this study, we have utilized MLPA to identify subtelomeric rearrangements in 7/124 (5.6%) individuals with mental retardation. The alterations detected are as follows: deletions of 22qter (twice), 2qter, 20pter and duplications of 4qter, 13qter and 18qter. Two independent MLPA kits from the same manufacturer with different probe recognition sequences confirmed these results. Furthermore, FISH and array-based CGH analysis to confirm the MLPA findings are underway and will be presented. One patient with an apparent 3pter deletion was shown to have a mutation in the probe recognition sequence on 3p. This points to one limitation of the assay and the specifics of this interesting case will also be presented. Overall, MLPA has performed very well and appears to be a valid methodology for testing cases of mental retardation with unknown etiology.

P0839. Familial Mediterranean Fever: 6 new mutations in Pyrin gene some of Dutch origin?

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Since many people of mediterranean origin live in the Netherlands, there is a growing demand for molecular diagnosis of FMF.
In 1998 we started to test for FMF mutations in exon 10 of the Pyrin/ Marenostrin gene. Although this yielded a number of known mutations, we had to expand the screening to all 10 exons because the overal yield was to low. Currently we sequence all 10 exons.
Up to now we have tested patients with complains of abominal pain and recurrent attacks of fever from over 200 families. In 130 of the families mutations were detected. FMF was definitely confirmed in over 60 families, in 17 families homozygosity for a mutation was detected and more then 40 were compound homozygous for mutations earlier reported. We also detected the E167D-F479L haplotype in combination with V726A to cause FMF, as a compound heterozygote. Further we detected the following 5 new (not yet reported) mutations:
Q97X; G304R; Q440N; K447N; Y688F;
We will discuss their possible role in FMF.

P0840. High sensitivity mutation detection in clinical samples using partial denaturation approaches by temperature gradient capillary array electrophoresis and denaturing liquid chromatography (dHPLC)

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We have employed two alternative approaches in separation of mutant homo- and hetero-duplex forms in DNA isolated from bioptic and resected tissue of colorectal tumors. The two techniques were 1. Temperature-Gradient Capillary Electrophoresis (TGCE) on a multicapillary sequencer and 2. Denaturing High-Performance Liquid Chromatography (dHPLC). The spectrum of samples investigated was ranging from early adenomas to invasive carcinomas and we have focused on selected mutant markers in most frequently mutated genes: k-ras, APC and p53. The optimized protocol included isolation of DNA followed by PCR amplification and subsequent separation of mutant forms. Results on selectivity and specificity will be presented for both techniques.

P0841. Regulation of Hermansky Pudlak Syndromes Genes in Human Lymphoblastoid Cell Lines

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Hermansky Pudlak Syndrome (HPS)[MIM#203300] is the major cause of albinism cases in Puerto Rico. HPS is caused by defects in several genes and seven of them have been identified in humans. *HPS2* codes for the b3A subunit of the AP3 adaptor complex and *HPS7* codes for dysbindin, a component of the dystrophin associated protein complex (DPC), the rest of the human genes are novel and show no sequence similarity to other proteins and have few

known functional domains. The HPS gene products are frequently undetectable in cells or tissues derived from both human patients and mouse models of HPS. The expression of the HPS1, *HPS2, HPS3, HPS4* genes in lymphoblast cell lines were examined by RT-PCR and Northern Blot. Analysis of the 5'-flanking DNA of several HPS genes using the TESS software showed potential response elements for glucocorticoid hormones, cAMP and Phorbol esters. Preliminary, Northern blot analysis show an inductive effect of Dexamethasone, cAMP and Insulin on the expression of *HPS2, HPS3, HPS4* genes. However, treatment with PMA caused a decrease in the mRNA^{HPS2} levels. Western Blot analysis of lymphoblastoid cells treated with PMA indicated a slightly reduction in the expression of HPS1 and HPS4 proteins in normal Lymphoblastoid cells. Further studies on the regulation of HPS genes are necessary to fully understand the cellular pathways affected in HPS. Grants from NIGMS S06GM08224 and R25GM61838 and NCRRG12RR03051 supported this study.

P0842. Medium-chain acyl-CoA dehydrogenase deficiency

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Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common inherited defect of fatty acid oxidation, characterized by episodes of illness in early childhood. The disorder may present after fasting with symptoms resembling Reye Syndrome, coma, hypoglycemia, hyperammonemia, fatty liver, and sudden death. Affected children may have only one episode of illness or multiple recurrences. A common mutation (985A >G) has been identified among patients with MCAD deficiency, which was found to have a carrier frequency of approximately 1:80 in the majority of Caucasian populations.
In this study, two unrelated MCAD patients, compound heterozygous for the 985A>G mutation, have been investigated. The diagnoses were based on the dried blood spot acylcarnitine profiles, which showed highly elevated octanoyl carnitine and decenoyl carnitine, indicating MCAD deficiency. To investigate the unknown mutation alleles, all twelve MCAD exons from patients' DNA were amplified and sequenced. Sequence analysis revealed two novel mutations, 250C>T and 244T insertion respectively.
In addition, in our total 41 patients with MCAD deficiency, including six patients detected by newborn screening using tandem mass spectrometry (MS/MS), the correlation of genotype to phenotype was also discussed.

P0843. MCDR1 Locus - Screening for candidate genes.

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MCDR1 is an autosomal dominant nonprogressive disease. It is characterized by bilaterally symmetrical but highly variable macular degeneration ranging from drusen to staphylomata. The peripheral retina can be variably abnormal. Light microscopy studies have demonstrated a discrete macular lesion characterized by focal absence of photoreceptors and retinal pigment epithelium with attenuation of the Bruch membrane and focal atrophy of the choriocapillaris. Adjacent to the macular lesion, some lipofuscin were also identified in the retinal pigment epithelium. We have carried out a candidate gene screening in families with MCDR1 for the GPR63 gene. GPR63 (belonging to a rodopsin like activity family of genes) is located within the locus for MCDR1 on 6q16 and involved in the pathway for G protein coupled receptors. The G protein coupled receptor genes are know to be involved in retinal diseases. Interacting transporter proteins like ABCA4 are also known to cause retinal disease. Direct sequencing of the entire gene coding and non coding was carried out on more than 12 probands representing different families. Although the gene represents a good candidate we did not observed any changes which segregated with the disease or was absent in control individuals. A 765 A>T change within the coding region of the gene did not segregate in the families and is considered a polymorphism. We rule out GPR63 as a candidate gene and present evidence to show that it is not directly involved in the etiology of the disease.

P0844. *SLC7A10* in 19q13 is not involved in the aetiology of cystinuria

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Cystinuria is an inherited metabolic disorder characterised by the abnormal urinary excretion of cystine and dibasic amino acids and results in the formation of cystine kidney stones. Two genes involved in cystinuria have been identified: Mutations in the *SLC3A1* (2p16) gene cause cystinuria type I, whereas mutations in the *SLC7A9* (19q13) gene can be detected in non-type I as well as in type I cystinuria patients. The mutation detection rate for both genes in cystinuric patients does not reach more than 80% and is influenced by several factors (screening techniques, ethnic origin, classification of patients). Therefore, the role of further genes in the aetiology of cystinuria has been postulated. Interestingly, linkage analysis in cystinuria families does so far not indicate the existence of more than the two cystinuria loci in 2p16 and 19q13. Thus, the localisation of further genes encoding amino acid transporter subunits within these regions is conceivable. One candidate is *ASC-1* (*SLC7A10*) in 19q13 which shows high homology with *SLC7A9*. To further elucidate whether *SLC7A10* is involved in the aetiology of cystinuria, we screened for mutations in two non-type I cystinuria families compatible with linkage with 19q13 but without detectable mutations in SLC7A9. Despite strong evidences for an involvement of *SLC7A10* in the aetiology of cystinuria, we could not identify any mutation in *SLC7A10* in the two families. Nevertheless, there remains the possibility that other genes are involved in cystinuria. Further molecular studies will clarify the complex nature of this disorder.

P0845. Inactivation of the spasmolytic trefoil peptide (Tff2) leads to increased expression of additional gastroprotective factors

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By defining a trefoil domain as a sequence of 38 or 39 amino acid residues with disulphide links between the intramolecular 6 cysteines the trefoil family (TFF) was assembled from several vertebrate peptides. The three mammalian TFFs are encoded by a three-gene-cluster. Trefoil factor family domain peptides, tissue-specific products of mucin-secreting epithelial cells, are thought to influence mucosal integrity. While *Tff1* and *Tff3* knock-out mice show serious functional disturbance in stomach and gut, *Tff2-/-* constructs do not display striking histologic effects. For a more *in-detail* study, we analysed possible differences in expression (wt vs. *Tff2-/-*) by applying a commercial DNA microarray (Affymetrix mouse chip, 12000 genes). When the threshold was set to >2, 62 genes were found up and 30 genes down-regulated. Since such microarray studies need to be evaluated with caution, solid confirmation of expression by Northern or quantitative PCR and protein analysis by Western blots or immunohistochemistry are required. Among the differentially expressed genes, we disregarded for the further study structural genes and those from the HLA group and concentrated on genes involved in immunoresponse. When qPCR measurements were normalised vs. 3 house keeping genes (*Gapdh, Gusb, Mhkg*) contradictory data eliminated *Timp, Mmp7* and *Gdp* but confirmed upregulation of three genes connected with the digestive tract: *Crip*, *Muc3* and *Tff3*. In summary, microarray analysis of the *Tff2* knockout shed new light on the role of Tff2 in maintaining homeostasis in gastrointestinal tract, pointing to its possible role in innate immunity.

P0846. RApidTRAP robot assisted TRAP assay

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Activation of telomerase plays a critical role in unlimited proliferation and immortalization of cells. Telomerase activity has been shown to correlate with tumor progression, indicating that tumors expressing this enzyme possess aggressive clinical behavior

and that telomerase activity may be a useful biomarker for early detection of cancer. However, measurements of telomerase activity by current methods are low-throughput and not robust enough to easily accommodate the required statistical analysis to determine if telomerase activity is a practical biomarker.
As part of the National Cancer Institute Early Detection Research Network, we have developed a robot assisted TRAP assay (RApidTRAP) of telomerase. Measurements of human telomerase reverse transcriptase catalytic subunit (hTERT) mRNA were performed in concert with measurements of telomerase activity. We determined hTERT mRNA concentration and telomerase activity in human normal (RPE-28) and cancer (A549) cell lines as well as in human serum. Telomerase activity measurements were made using the TRAP/PCR CE method on 50 cells / reaction from cell extracts. Measurement of hTERT mRNA was made using specific primers on a LightCycler in the range of 10 cells / reaction. Using this combination of telomerase activity and hTERT mRNA measurements, the automated system improved efficiency over traditional methods. Large-scale studies are required before telomerase activity becomes a practical biomarker for use in clinical decisions regarding patient management. Such studies will only be possible using automated systems with lower cost. The use of RApidTRAP results in at least a four-fold improvement in throughput and cost.

P0847. In-depth detection of somatic mosaicism with a combined mutation scanning strategy that utilizes Surveyor™ mismatch endonuclease and WAVE®-HS DHPLC

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Both Mendelian and non-Mendelian disorders are associated with somatic mosaicism, with the most studied examples of being found in the pathogenesis of cancer. The failure to identify mutations associated with somatic mosaicism can lead to inaccurate reporting of mutation frequencies and missed genetic diagnoses. These considerations are also very apparent in the detection of somatic mutations in cancerous tissues and disease-causing mutations in heteroplasmic mitochondrial DNA. We describe a thorough approach for the identification of somatic mosaicism that combines the simplicity of Surveyor™ mismatch endonuclease technology with the high sensitivity detection capabilities of the WAVE® HS nucleic acid fragment analysis system. Surveyor nuclease mutation detection is based on an enzymatic method for detecting DNA heteroduplexes and can be used to detect known and unknown mutations including all base substitutions as well as insertions and deletions. The WAVE HS System utilizes post-separation fluorescent dye intercalation for the high sensitivity detection of nucleic acids containing single base substitutions, small deletions or insertions. By combining these two technologies, we were able to scan efficiently a large number of genes for somatic variations within different patient cohorts. Examples will be presented in which somatic mosaicism was identified with this in-depth scanning approach; these include mutations in APC, TP53, PTEN, TSC1, TSC2, NF1, NF2, VHL, WASP and mitochondrial DNA, demonstrating the detection and identification of somatic genetic variants that are below 2% of the total alleles present. These somatic mutations, which result in functional aberrations within the coding regions of the analyzed genes, were not detectable using traditional mutation detection approaches, such as DNA sequencing.

P0848. High Sensitivity Fluorescence Detection of Double-Stranded DNA using the WAVE® HS High Sensitivity System under Non-denaturing Conditions

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Intercalation and groove binding dye chemistries coupled with high efficiency size-based separation technology provides enhanced sensitivity compared to direct UV detection for the analysis of double-stranded DNA (dsDNA) under non-denaturing conditions. The WAVE HS High Sensitivity System consists of a post cartridge reactor where the effluent of the cartridge, or in the case of monitoring both the absorbance and fluorescence, the effluent of the absorbance detector, is mixed with a dye solution which is introduced via a pump that provides pulseless delivery. The fluorescence detector monitors the resulting cartridge effluent/dye solution mixture. Intercalation and grove binding dyes are an attractive solution for increased sensitivity in fluorescent dsDNA analysis since fluorescence only occurs when bound to the polynucleotide; therefore, the background noise is minimal. In addition, non-covalent fluorescent labeling of DNA using intercalation dyes has an advantage over fluorescently labeled primers since multiple dye molecules can bind to each polynucleotide; but, with a fluorescently labeled primer, each polynucleotide has only a single fluorophore. Introducing the dye solution post cartridge minimizes system/cartridge contamination. When analyzing dsDNA under non-denaturing conditions, we have determined that the sensitivity enhancement of the fluorescence detection system using WAVE Optimized HS Staining Solution I is greater than 200 as compared to direct UV detection. We explore linearity and limits of detection as compared to direct UV detection for a variety of DNA sizing assays.

P0849. Deletion analysis of the SMN and NAIP genes in 60 Tunisian patients with Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is an autosomal recessive motor neuropathy characterised by selective degeneration of anterior horn cells of the spinal cord. Childhood SMA is divided into three types (I-III) on the bases of age of onset and severity. These disorders have been linked to 5q13 region, where mutations in the survival motor neuron1 (SMN1) gene have been found in affected individuals. In the cases of adult-onset SMA (type IV), reports of homozygous absence of SMN1 gene have been rare. We conducted deletion analysis of SMN and neighbouring gene, NAIP (neuronal apoptosis inhibiting protein). Among 60SMA patients (35typesI, 16TypesII, 8TypesIII and 1type IV), all of Tunisian origin, exon 7 of the SMN1 gene was homozygously absent in 100% of type I, 81% of type II, 75% of type III and in the case of type IV SMA patients. Deletion of SMN1 exon 8 was detected in 91% of type I, 87,5% of type II, 75% of type III and in the case of type IV SMA patients. All control individuals who were studied had normal SMN1 genes. All control individuals but one have exon 5 NAIP deletion. While NAIP is commonly deleted in SMA, this is unlikely to affect disease severity; it was deleted in 5 patients with mild phenotypes (type III) and present in 8 patient with severe phenotype (type I). In conclusion, our results are in agreement with the general consensus that there is no correlation between genotype and phenotypic expression of the disease.

P0850. Qualitative and quantitative analysis of mRNA associated with splicing mutations in the CFTR gene with the aim of developing a CF splicing chip -Clinical phenotypes of patients

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Splicing mutations result in relatively large cDNA differences between normal and CF carriers/patients thus providing an opportunity to discriminate between them using differential hybridization. Seven splicing mutations of the *CFTR* gene were investigated by RT-PCR analysis of mRNA extracted from nasal epithelial cells. The cDNA products were electrophoresed on a 6% acrylamide denaturing gel. Relative amounts of mutant and normal cDNA products were sized and quantitated while aberrant splicing products were sequenced. We studied seventeen patients with 2789+5G>A, seven with 711+3A>G, four with 621+3A>G, two with 2751+2T>A, two with D565G, 2 with 1525-1G>A, and one with 296+1G>C. mRNA analysis showed that mutations which disrupt the 5' splice donor sites of introns result in skipping of the upstream exon as a major product. Mutation 621+3A>G showed an additional product containing part of exon 4, resulting from activation of a cryptic donor splice site. 1525-1G>A

which disrupts the 3' splice acceptor site of intron 9 results in skipping of the downstream exon 10 as a major product and a minor product resulting from activation of an alternative splice site within exon 10. D565G disrupts the exonic splicing enhancer sequences of exon 12, and leads to the production of aberrantly spliced mRNA with omission of exon 12. Design of exon n-exon n+2 junction (single exon skipping) probes should detect the majority of these splicing defects. Splicing mutations with severe deficiency of normal *CFTR* mRNA result in severe phenotype, while mutations with higher residual levels of normal *CFTR* mRNA result in a milder phenotype.

P0851. Analysis of gene conversions in the patients with the non-classical form of steroid 21-hydroxylase deficiency

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Congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency is a common inherited defect of adrenal steroid hormone biosynthesis. Unusually for genetic disorders, the majority of mutations causing CAH apparently result from recombination between the CYP21 gene encoding the 21-hydroxylase enzyme and the closely linked, highly homologous pseudogene CYP21P.
The frequency of the major mutations (P30L, del8, delB, I2splice, I172N, V237G, V281L, Q318X, R356G and P453S) was studied in 70 Russian patients with the non-classical form (NC) of CAH caused by 21-hydroxylase deficiency. Only 25% of the CAH chromosomes have been identified. The search for large conversions in the CYP21 gene of 10 NC CAH women was undertaken. A novel CYP21/CYP21P hybrid gene was identified in 2 patients.
This newly discovered CYP21/21P hybrid gene is characterized by the junction site close to exon 8 and its 3'-region corresponding to this one of CYP21P pseudogene. One more woman of the same group had also V281L mutation in 7-th exon of this hybrid gene. This mutation is common in patients with non-classical form of CAH. Thus new type of mutation which results in hybrid gene might contribute to non-classical form of CAH.

P0852. Genes PARK1 and PARK2 analyses in Parkinson disease patients from the Volga-Ural region

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Parkinson disease (PD) is a neurodegenerative disorder, characterized by selective degeneration of dopaminergic neurons within the substantia nigra. The etiology of idiopathic PD is still undefined, but it is considered that PD is due to genetic susceptibility factors, interacting with environmental exposures.
We studied 200 patients aged 48-83 years (mean 62 years) with PD from the Volga-Ural region. All patients showed at least two of the four principal signs of PD (resting tremor, rigidity, bradykinesia, postural-reflexes impairment) and had no clinical features of any other parkinsonian syndrome.
The 200 control subjects were collected according their age, ethnic origin, gender, area of residence. Controls demonstrated no significant signs of cognitive or neurological impairment.
Using SSCP- analyses of all 12 exons of PARK2, followed by sequencing of shifted exons, we have found out Ser167Asn in the 4th exon and Asp394Asn in the 11th exon in 2% of PD patients. Such mutations were not found in controls. In the 2 and 4 introns we detected +25 T-> C transition and -25Adel respectively.
Besides, we've found heterozygous 12 exon deletion (Trp450Stop) in 2 unrelated patients of Russian origin, which wasn't described earlier.
We've analyzed A53T and A30P mutations in PARK1, but not a single PD patients had such mutations. It proves, that PARK1 mutations is a very rare course of PD development in patients from the Volga-Ural region.
So, PARK2 structure disturbance can result in affected proteins with loss of function and PARK2 mutations may cause Parkinson disease development.

P0853. Mutations in Ligase IV resulting in a Nijmegen Breakage Syndrome-like phenotype

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Nijmegen Breakage Syndrome (NBS) is an autosomal recessive condition, characterized by microcephaly, growth retardation, characteristic facial features, recurrent infections and an increased risk of malignancy. The clinical phenotype of Ligase IV Syndrome, a rare autosomal recessive condition caused by mutations in the LIG4 gene, closely resembles that of NBS. We are reporting a 4 ½-year-old boy who presented with acute T-cell leukemia. A genetics referral was made for evaluation of his microcephaly, dysmorphic facies, short stature and developmental delay. The facial gestalt was strongly reminiscent of NBS. The patient died shortly after the onset of treatment for his T-cell leukemia. Subsequently chromosome breakage study results showed a high rate of breakage in a fibroblast culture. A colony survival assay was done to assess radiosensitivity; the results showed radiosensitivity at the high end of that seen in NBS. Mutation screening for the NBS1 gene was negative. Sequencing of the LIG4 gene showed that the patient was homozygous for the 2440 C>T mutation, resulting in a truncating mutation R814X. Two patients reported by O'Driscoll et al. (2001) were compound heterozygotes for this truncating mutation, but this patient is the first to be homozygous for this mutation. We will review the clinical features of this interesting patient, review the other reported patients in the literature and present the molecular data.

P0854. An investigation of the interleukin-6 -174G/C polymorphism in ischaemic heart disease using family based tests of association

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Introduction. Atherosclerosis is an inflammatory disease. Interleukin-6 (IL6) is a major pro-inflammatory cytokine that has been linked to the development and progression of ischaemic heart disease (IHD). A single nucleotide polymorphism with functional importance has been identified in the promoter region of the IL6 gene at position -174 (-174G/C polymorphism). This polymorphism may affect the transcription of IL6 and as such is a plausible genetic risk factor for IHD.
Aims. To investigate for the presence of linkage disequilibrium between the IL6 -174G/C polymorphism and premature onset IHD using two family based tests of association.
Methods. Families with at least one individual with premature onset IHD (males ≤55 years, females ≤60 years) with all grandparents born in Ireland were recruited. Genotyping was performed using a PCR-RFLP method. Analysis was performed using the combined transmission disequilibrium test (TDT)/sib-TDT and the pedigree disequilibrium test (PDT). These tests are unaffected by population admixture.
Results. 388 families consisting of 1023 individuals (418 affected cases, 110 parents, and 495 unaffected siblings older than the proband was at IHD onset) were recruited and genotyped. The -174G/C polymorphism was demonstrated to be not associated with premature onset IHD by either the combined TDT/sib-TDT (p=0.16) or the PDT (p=0.12). The study afforded over 80% power to detect a deviation of allele transmission from 50 to 60 %.
Conclusion. The IL6 -174G/C polymorphism is not associated with premature-onset IHD in the Irish population.

P0855. Genome-wide search for asthma loci in a German multiplex family

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Asthma is one of the most frequently investigated complex diseases. Numerous genome-wide scans have been conducted and many genes with small effect could already be identified. Nevertheless - an understanding of pathophysiological mechanisms as a final step of genetic analysis is not yet in sight.
We conducted a genome-wide scan with 371 microsatellite markers in a German multiplex family. As the inheritance in this family seems to be in accordance with a monogenetic trait, we assumed that only one or few genes are responsible for their asthmatic phenotype.
17 individuals could be analyzed of which 8 suffered from severe asthma.
The highest NPL score of 4.3 (p=0.000065) was reached at marker D5S422 on chromosome 5q. For this marker and an adjacent marker 5 cM apart all affected individuals shared one haplotype and the unaffected sibling did not.
Loci on chromosome 5q have already been identified in several linkage studies - our family offers the encouraging opportunity to define the underlying genetic variation.
This approach based on one large family with many affected individuals might open new roads for complex human diseases whereas only little genotyping effort is needed.

P0856. Association of the dopamine transporter gene (DAT1) core promoter polymorphism -67T variant with schizophrenia

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Dysfunction of the central dopaminergic neurotransmission has been suggested to play an important role in the etiology of schizophrenia. The dopamine transporter (DAT1) mediates the active reuptake of dopamine from the synapses and thereby plays a key role in the regulation of the dopaminergic neurotransmission. In this study, we sought to determine the possible association of the DAT1 gene core promoter polymorphism -67A/T with schizophrenia in a case/control study. The allele and genotype frequencies of the polymorphism were studied in 100 patients and 100 controls, which were matched on the basis of sex, age and ethnicity. The genotype frequencies in the patients group were as follows: AA 29%; AT 59%; TT 12% vs. the genotype frequencies in the control group: AA 57%; AT 38%; TT 5% (χ^2=16.54, df = 2, $p \leq 0.0003$). For the first time, these findings provide evidence for the contribution of the DAT1 gene core promoter polymorphism to the etiopathophysiology of schizophrenia at least in the Iranian population that we studied.

P0857. Promoter SNPs of the interleukin-6 gene are associated with type 2 diabetes

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Elevated blood concentrations of interleukin (IL)-6 are associated with type 2 diabetes (T2D). Since the impact of IL-6 gene single nucleotide polymorphisms (SNPs) on diabetes status, metabolic and inflammatory parameters have not been previously analyzed in a population-based study, we investigated the impact of three promoter SNPs of *IL-6* (A–598G, C-573G and C–174G) in 704 elderly participants of the KORA Survey 2000 (230 patients with T2D, 235 subjects with impaired glucose tolerance/IGT and 239 controls). The rare –573C allele was significantly associated with T2D. Heterozygous carriers of the C allele had higher levels of circulating IL-6. This increase was significant in women and in lean to moderately overweight subjects (BMI ≤ study median 28.7). The –598G and –174G alleles were in strong linkage disequilibrium and significantly associated with T2D. The association was stronger in men and again in leaner subjects (BMI ≤ 28.7). Circulating IL-6 levels were not affected, but significantly elevated levels of the chemokine CCL2/MCP-1 in carriers of the protective genotypes indicated an indirect effect of these SNPs. None of the SNPs showed association with IGT.
Our findings confirm that SNPs located in the promoter of IL-6 can be considered as risk factors in the etiology of T2D. The contribution of IL-6 SNPs can be either direct by increasing the level of circulating IL-6 or indirect by influencing the levels of other immune mediators such as CCL2/MCP-1 which might exert protective or pathological effects. Sub-analyses showed that the impact of IL-6 SNPs depends on sex and on obesity.

P0858. Genetically heterogeneous selective intestinal malabsorption of vitamin B_{12}: founder effects, consanguinity, and high clinical awareness explain aggregations in Scandinavia and the Middle East

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Selective intestinal malabsorption of vitamin B_{12} causing juvenile megaloblastic anemia (MGA; Imerslund-Gräsbeck disease, MIM 261100) is a recessively inherited disorder that is believed to be rare except for notable clusters of cases in Finland, Norway, and the Eastern Mediterranean region. The disease can be caused by mutations in either the cubilin (*CUBN*; MIM 602997) or the amnionless (*AMN*; MIM 605799) gene that form a receptor complex called cubam. Only some 230 cases have been described in the literature in the past 44 years. We studied 48 sibships and found all cases in Finland to be due to *CUBN* (3 different mutations), all cases in Norway to be due to *AMN* (2 different mutations), while in Turkey, Israel, and Saudi Arabia there were 2 different *AMN* mutations and 3 different *CUBN* mutations. Interestingly, haplotype evidence excluded both *CUBN* and *AMN* conclusively in 5 families and tentatively in 3 families, suggesting the presence of at least one more gene locus that can cause MGA. We are currently working towards identifying this putative locus. We conclude that the Scandinavian cases are typical examples of enrichment by founder effects while in the Mediterranean region high degrees of consanguinity expose rare mutations in both genes. We suggest that in both regions, physician awareness of this disease causes it to be more readily diagnosed than elsewhere; thus, it may well be more common worldwide than previously thought. Several recently diagnosed cases in Europe and the United States support this hypothesis.

P0859. CCM2 mutations account for 13% of cases in a large collection of kindreds with hereditary cavernous malformations

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Cerebral Cavernous Malformations (CCM) are vascular malformations characterized by abnormally enlarged capillary cavities without intervening brain parenchyma that are found in 0.1-0.5% of the general population. Recently, mutations in a gene called *MGC4607* have been found in CCM families linked to the *CCM2* locus. We have screened the *CCM2* gene in 21 families that are taking part in the IFCAS. None of these families have a *CCM1* mutation.
The *CCM2* gene contains 10 coding exons. It codes for the malcavernin protein, which has a predicted Phosphotyrosine Binding Domain (PTB). By dHPLC screening and sequencing variants, we have found three novel mutations segregating in four families. Two invariant splicing mutations were found in exon 1 and 3. A nonsense

mutation was found in exon 2 which segregated with two families of European descent. All mutations predict to lead to a shorter protein with deletion of all or part of the PTB.
Krit1, the *CCM1* gene product, was recently shown to bind to a PTB containing protein called icap1α, which also binds to the β1-integrin cytoplasmic domain. Hence, malcavernin may be interacting with a binding partner of Krit1, indicating a shared pathway.
So far, 45 families have been screened for mutations in *CCM1* and *CCM2*. Twenty-two of these families segregate a *CCM1* mutation, while 6 families contain a *CCM2* mutation. There remain 17 families with no *CCM1* or *CCM2* mutations but which will almost certainly have mutations in the non-identified *CCM3* gene, and possibly in some as yet unidentified CCM locus.

P0860. Autosomal Dominant Sensory Ataxia maps to chromosome 8p12-8q12.1

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The ataxias are composed of a heterogeneous group of disorders that share the hallmark symptom of gait difficulty, but also may present with retinopathies, spasticity, neuropathy, deafness and/or motor neuron disease. A New Brunswick family of Anglo-Saxon origin has been identified with variable pyramidal weakness and sensation and reflex loss in the extremities. The impairment of preganglionic sensory nerve fibers leads to a loss of proprioception below the knees and causes ataxia. Despite this, they do not display overt signs of a peripheral motor or sensory neuropathy. As a result, this novel disorder has been entitled Autosomal Dominant Sensory Ataxia (ADSA). DNA from nine affected members was collected and subjected to a 400-marker genome scan. Markers around a positive region on chromosome 8 were analyzed and yielded a maximum LOD-score of 4.90 at θ = 0 for the marker D8S1791. The novel ADSA locus spans 9.1cM (24.9Mb) between the markers D8S601 and GATA156H01. This locus has been designated Sensory Ataxia 1 (SNAX1) and maps to chromosome 8p12-8q12.1. A number of interesting candidate genes in the region have been screened, including one with homology to tetrahydrofolate synthase, without detection of a causative mutation. Detection of a mutation responsible for ADSA would provide a useful connection between this disease and similar ataxias and neuropathies.

P0861. Interleukin (IL)-1 β, IL-1 receptor antagonist, IL-6, IL-8, IL-10 and tumor necrosis factor α gene polymorphisms in patients with febrile seizures

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Background: Inflammation and genetics may play a role in the pathogenesis of febrile seizures (FSs). It is becoming apparent that the endogenous pyrogens affect neuronal excitability and provides a putative common link between fever and seizure activity. The study aim was to test whether interleukin-1β(IL-1β), IL-1 receptor antagonist (IL-1 Ra), IL-6, IL-8, IL-10 or tumor necrosis factor (TNF-α) gene polymorphisms could be used as markers of susceptibility in FSs Methods: By performing an association study, we used single nucleotide polymorphisms to investigate the distribution of genotypes of these cytokines in patients with FSs. A total of 104 patients with FSs and 143 normal control subjects were included. IL-1βpromoter, IL-1βexon 5, IL-1 Ra, IL-6, IL-8, IL-10 and TNF-α gene polymorphisms were identified by polymerase chain reaction-based restriction analysis. Results: There was no significant difference in the distribution of genotypes and allelic frequencies between both groups for IL-1βpromoter, IL-1βexon 5, IL-6 promoter, IL-8 and TNF-αgene polymorphisms. In contrast, the genotypes of IL-1 Ra, and IL-10 were significant different between both groups (P=0.028 and P<0.0001, respectively). Conclusions: These data suggest that the IL-Ra and IL10 genes or a closely linked gene might be one of the susceptibility factors for FSs. Further studies could be focused on the analysis of IL-Ra and IL10 RNA and protein in children with FSs.

P0862. Elaboration and usage of AS-PCR method for detection the Sp1 polymorphysm in collagen type I α1 gene, *Bsm*I polymorphism in vitamin D receptor gene, and XbaI and PvuII polymorphisms in estrogen receptor α gene in women with postmenopausal osteoporosis.

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Osteoporosis is characterized by low bone mineral density (BMD), deterioration of the microarchitecture of bone, and subsequent increased fracture. BMD has a strong genetic component. Polymorphisms in collagen type I α1 (ColIα1) gene, vitamin D receptor (VDR) gene, and estrogen receptor (ER) α gene are most often examined polymorphisms in relation to bone mineral density (BMD) and fracture risk. Commonly used method of these polymorphisms detection is a restriction fragment length polymorphisms (RFLP) method. We developed a new allele-specific PCR (AS-PCR) based method, that possess several advantages in comparison with RFLP. It is more robust, simple and reliable because a stage of restriction endonuclease cleavage is excluded. In proposed method the analysis of PCR fragments is performed automatically using capillary electrophoresis on MultiGene device (ATG-Biotech, Russia). Fluorescent detection allows to avoid the ethidium bromide usage.
The primers, conditions of AS-PCR and analysis of amplified DNA fragments for fast and reliable detection of Sp1 ColIα1, BsmI VDR, XbaI and PvuII ERα gene polymorphisms were elaborated. The blood samples of 66 patients (women of age 60-75 with different stages of osteoporosis) were analyzed. The results obtained were confirmed by direct DNA sequencing.
Conclusion: The new AS-PCR-based method for detection of Sp1 ColIα1, BsmI VDR, XbaI and PvuII ERα gene polymorphisms was developed, which can be used for large-scale polymorphisms analysis.

P0863. Chromosome 1 association map to identify gene(s) associated with age-at-onset and risk for Parkinson disease

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We previously reported genetic linkage of a locus controlling age-at-onset (AAO) in Parkinson disease (PD) to a region on chromosome 1p (LOD=3.41)[1]. This region overlaps with the risk linkage peak for PD in the Icelandic study (LOD=4.9)[2]. To identify the gene(s) that are associated with AAO and risk in this region, we performed an association map study where we genotyped single nucleotide polymorphisms at an average distance of 100 kilobases over a 20 megabase region under the AAO linkage peak. Our dataset consists of 284 multiplex families with 574 affected and 641 unaffected individuals. The average AAO±SD of affected individuals is 59.9±12.7 yrs (range: 14-90 yrs; 58.3% males). The disease status and AAO were the traits of interest. Using the Pedigree Disequilibrium Test, we tested whether a marker is associated with the risk of getting PD. For association with AAO, we applied the orthogonal model and Monks–Kaplan method implemented in the Quantitative Trait Disequilibrium Test program. We have identified several markers associated with either risk or AAO, or both. Among them, several genes showed strong association and have promising biological implications for PD. We are currently genotyping additional polymorphisms in these genes.
1. Li YJ et al (2002). Am J Hum Genet 70:985-993.
2. Hicks AA et al (2002). Ann Neurol 52:549-555.

P0864. Vitamin D receptor polymorphism is risk factor for development of type 1 diabetes mellitus

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The polygenetic susceptibility to type 1 diabetes (T1DM) is well established and recent studies have demonstrated linkage of a

vitamin D receptor gene (VDR), interleukin-1 receptor type 1 gene (IL1R1) and Neuro D/BETA2, a transcription factor of the insulin gene, to disease. We studied 134 individuals with T1DM and 132 control subjects. The genotyping was performed using PCR and BsmI, ApaI and TaqI restriction enzymes for VDR polymorphism, PstI, HinfI and AluI for IL1R1 polymorphism and MwoI for NeuroD/BETA2 polymorphism. Data were analysed using the chi-square test. VDR gene polymorphisms are associated with an increased risk of T1DM in Dalmatian population caused with differences in Taq I genotype distribution between T1DM and control subjects. The genotype combination, which conferred strongest susceptibility to T1DM, was BBAAtt (P=0.002). Interestingly, the BAt haplotype was found to be a risk factor in a German population, the only European population tested thus far. All tested polymorphisms of IL1R1 gene and NeuroD/BETA2 gene did not display difference among case and control subjects. Our data implement involvement of vitamin D in pathogenesis of T1DM.

P0865. A genetic polymorphism in OPRM1 gene is associated wth bipolar disorder and major depression

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Recent data from animal experiments suggest that functional polymorphisms in μ- and δ-opioid receptor genes in humans may play a role in anxiety and depression. Moreover, there exists some evidence that μ-opioid receptor (OPRM1) agonists have antidepressant activity in patients with severe depression. Preliminary studies of OPRM1 gene polymorphisms have shown association with drug addiction and alcohol abuse. We have performed an association and haplotype analysis of genetic variants in OPRM1 gene by genotyping 14 single nucleotide polymorphisms (SNPs) with Arrayed Primer EXtension (APEX) technology. Altogether 224 unrelated patients, among them 177 with unipolar major depression and 47 patients with bipolar disorder, and 160 healthy control subjects from Estonian population were recruited in this study. Association analysis revealed significant differences in the distribution of allele frequencies of the common missense variant 118A-G (N40D) between cases and controls, with significantly lower frequencies of 118G allele in affected individuals. We demonstrated significant allelic and genotypic association for bipolar disorder (P=0.007) and major depression without comorbid conditions (P=0.03). Haplotype analysis demonstrated that the OPRM1 haplotype GGC, carrying protective 118G allele, was associated with lower risk for bipolar disorder (OR=0.37; P=0.019) compared to the major reference haplotype (AGC), confirming the positive association between marker 118A-G and bipolar disorder. Our results give support to the possible role of genomic variations in OPRM1 gene in genetic predisposition to affective disorders.

P0866. Differential fertility associated with common apolipoprotein E (APOE) alleles: a study in industrial and pre-industrial populations.

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The relationships between APOE genotypes and number of liveborn children were analyzed in 160 Italian subjects at the postreproductive age, 124 African-Ecuadorians and 40 Native Americans (Cayapa Indians). In the Italian sample (women born between 1917 and 1921, fertility rate = 3.7) the highest number of children was found associated with *e*3/e*3* genotype. Moreover the mean number of children of *e*2* allele carriers was significantly lower than that of *e*3/e*3* and *e*4/e*3* subjects and a clear inverse relationship between number of children and *e*2* carrying genotype frequency was found. In African-Ecuadorians and in the Cayapa (fertility rates = 6.5 and 6.2 respectively), the highest number of children was found to be associated with the *e*4/e*3* genotype; the *e*4/e*3* genotype frequency in the African Ecuadorian women with 9-17 children, was about three times that of the women with 0-8 children. The discrepancies in the APOE polymorphism and fertility relationships observed in the industrial and pre-industrial populations could be explained by the different functional properties of the APOE isoforms and their interactions with different environmental conditions including not only dietary habits, but also reproductive strategies (number of children, age at first birth, stable/unstable matings, birth interval etc.)

P0867. Ethanol -metabolizing enzymes polymorphisms in male Russian patients with alcoholic liver disease

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Alcoholism is an important cause of chronic liver disease, but alcoholic cirrhosis develops only in 10% - 15% of individuals. Alcohol is oxidized initially to acetaldehyde, principally by alcohol dehydrogenase (ADH) and cytochrome P450 (CYP2E1), and then to acetate by aldehyde dehydrogenase (ALDH). Polymorphisms of the genes encoding of ethanol-metabolizing enzymes alcohol dehydrogenase (ADH1B), aldehyde dehydrogenase (ALDH2) and cytochrome P-450 (CYP2E1) are in association with inter-individual difference in alcohol metabolism and susceptibility to alcoholic liver disease (ALD). The aims of this study were to determine genotypes and alleles frequencies of ADH1B, ALDH2 and CYP2E1 genes in male Russian patients with ALD (n=126) and healthy control group (n=120). We have found higher frequencies of homozygous genotypes for ALDH2 (c2 = 3,74; p<0,05; OR= 5,02) and higher frequencies of heterozygous genotypes for CYP2E1 (c2 = 7,52; p<0,05; OR=7,37) genes in male Russian patients with ALD. So, we can assume that polymorphic variants of these genes play an important role in the development of ALD.

P0868. Clinical and molecular findings in nine large Cypriot families with Focal Segmental Glomerulosclerosis

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Focal Segmental Glomerulosclerosis (FSGS) is a common non-specific renal lesion characterized by proteinuria and progressive decline in renal function, that can be of idiopathic or familial nature. For the familial form, recessive and dominant inheritance has been recognized. Two recessive loci have been mapped and the respective genes cloned, coding for nephrin and podocin on chromosomes 19q13.1 and 1q25-32 respectively. For the dominant form two loci have been mapped. The ACTN4 gene on chromosome 19q13 that encodes á-actinin-4 has been studied and three mutations have been identified in respective families, whereas the second gene has been mapped to 11q22-24 but is not cloned yet. In Cyprus we located 9 multigeneration families with autosomal dominant inheritance. There are many affected members 12 of whom were ascertained by percutaneous renal biopsies. We performed linkage analysis with markers spanning the previously reported critical FSGS region: D19S412, D19S425, D19S191, D19S223, D19S417, D19S228 and D19S190. We showed that at least three of the families link to 19q13. We directly sequenced the entire coding region of the reverse transcribed ACTN4 gene from affected and healthy members of these families. We did not identify any pathogenic mutations. It is probable that the approach we used for mutation identification missed existing mutations, such as large gene deletions or other rearrangements. However, it is equally likely that another gene exists in or near the critical region we examined by linkage analysis which harbors the responsible pathogenic defects. Such candidate is the WT1IP recently characterized that demonstrates podocyte expression.

P0869. Insertion/deletion polymorphism of the ACE gene and adherence to ACE-inhibitors

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Background: Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients.
Objective: To investigate whether the insertion/deletion (I/D) polymorphism of the ACE gene modifies the response to ACE-inhibitors (the average dose of an ACE-inhibitor, or discontinuation of an ACE-inhibitor, or addition of other antihypertensive drug class).
Methods: We used data from the Rotterdam Study, a population-based prospective cohort study in the Netherlands, which started in 1990 and included 7,983 subjects of 55 years and older. During follow-up, 252 of the 1,480 persons who used ACE-inhibitors were identified as starters. Starters were classified as subjects who did not use antihypertensive drugs between 1 Jan until 31 May 1991. The rate of discontinuation and addition was determined with a Cox proportional hazard model. The average dose of ACE-inhibitors was assessed in all subjects who used ACE-inhibitors during 1 Jan 1991 and 31 Dec 1999. We used ANOVA and t-test statistics to compare the average dosages between ACE genotypes.
Results: Results indicated that there was no significant difference between people with DD, ID or II genotype in the rate of discontinuation or addition (DD versus II; RR=1.23, 0.83-1.81 and ID versus II; RR=1.07, 0.75-1.51). For all ACE-inhibitors users the average prescribed daily dose was 1.02 ± 0.75, 1.04 ± 0.74, 1.04 ± 0.76 for DD, ID, and II genotype,(overall p-value=0.85).
Conclusion: The I/D polymorphism of the ACE gene played no role in the average prescribed dose, and discontinuation, or addition.

P0870. Analysis of variants in the *CAPN10* gene and their association with type 2 diabetes

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Type 2 diabetes mellitus (T2DM) is a typical complex genetic disease. After a genomewide scan followed by a positional cloning approach the Calpain-10 gene *(CAPN10)* on chromosome 2q37.3 was identified as a susceptibility gene for T2DM. *CAPN10* encodes a ubiquitously expressed cysteine protease, essential for calcium-regulated intracellular signalling. It plays a crucial role in insulin secretion and action. However, association studies between *CAPN10* and T2DM produced controversial results.
Therefore, we analysed selected SNPs in the *CAPN10* gene, previously associated with T2DM. Genotyping was based on the MALDI TOF MS technology.
Association of 7 SNPs with T2DM was tested among 254 probands with T2DM, 248 probands with impaired glucose tolerance and 250 age and gender matched control subjects from KORA S2000, a German population-based study. Statistical analysis did not reveal any significant association between the analysed polymorphisms and the different states of T2DM, neither individually nor as haplotypes. Recently, SNP-44 located in a transcription enhancer element next to the common SNP-43 turned out to be a promising candidate variant. It is in perfect linkage disequilibrium (LD) with the missense mutation Thr504Ala (SNP-110) and two polymorphisms in the 5`UTR (SNP-134 and SNP-135). Therefore, a meta-analysis with seventeen case/control studies, including KORA S2000, has already been published. The SNP-44 C allele was shown to be associated with an increased risk of T2DM (OR=1.17, P=0.0007). These results suggest that either the SNP-44 or a SNP in high LD may contribute to T2DM susceptibility.

P0871. SNP-44 in the calpain 10 gene is associated with waist-to-hip ratio in probands of the Augsburg Diabetes Family Study

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Aims. Variants of the gene *CAPN10*, which codes for the cysteine protease Calpain 10, have been shown to be associated with Type 2 diabetes mellitus (T2DM) in several but not all studies. The present study investigated the association of the *CAPN10* polymorphisms *SNP-44* (*CAPN10-g.4841T/C*; *T = allele 1*), *SNP-43* (*CAPN10-g.4852G/A*), *ins/del-19* (*CAPN10-g.7920indel32bp*), and *SNP-63* (*CAPN10-g.16378C/T*) individually and as haplotypes with T2DM, and with (central) obesity in families from Augsburg (Southern Germany).
Subjects/Methods. The study population consisted of 580 families of the Augsburg Diabetes Family Study (ADFS) with 1587 individuals, of whom 1393 were extensively phenotyped. Families were ascertained through an index proband with known T2DM, who had at least one full sib or both parents willing to participate in the study. Family-based association tests were performed, when possible with adjustment for covariates.
Results. No association was found between the four *CAPN10* variants tested individually or as haplotypes and T2DM. However, a nominally significant association of *SNP-44* with waist-to-hip ratio (WHR) was found, *allele 2* being associated with a considerably higher WHR estimate after adjustment for sex, age and body-mass-index: the WHR increased by 0.01 per one copy of allele 2 (p=0.04), which corresponds to one quarter of the mean difference in WHR between T2DM sibs and sibs with normal glucose regulation. Further adjustment yielded a p-value of 0.024.
Interpretation. We conclude that association of *SNP-44* with WHR could be the underlying cause of the association of this variant with T2DM, which was detected in different studies.

P0872. The first experience of acetylator phenotype research among patients with congenital glaucoma

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Association of the acetylator polymorphism (AAC2, MIM243400, gene map locus 8p23.1-p21.3) with different disorders has been demonstrated by several authors, but acetylator phenotypes in congenital glaucoma have not been studied. We investigated ‘rapid inactivation’ versus ‘slow inactivation’ among patients with congenital glaucoma.
Methods: acetylation of Sulfamethazine was determined with the methods described by G. Evans (1969) and O. Bulovskaya (1990). The identification of recessive Slow/Slow homozygotes was realized as described by M. Mkheidze (1999). The cut-off for Slow/Slow phenotypes was found to be below 37% and the mean value was 20%. Materials: a cohort of patients with congenital glaucoma (21, aged from 8 to 14 yr) Results. All patients were found to have the Slow/Slow phenotype with low enzyme activity (14-18%). The frequency of this phenotype is 0.549 for St. Petersburg population (M. Mkheidze, 1997). Expected number of the patients with Rapid phenotype would be at least 8-11 persons. Rapid cicatrization of aqueous humor outflow is the most frequent cause of unsuccessful results after glaucoma operations particularly among children.
Our suggest that the lower enzyme activity the higher the rate of cicatrization on the place of surgical interference. Investigation of AAC2 polymorphism among patients with congenital glaucoma may be a helpful parameter for the correct prognosis and predictive counselling

P0873. MTHFR 677TT genotype may be a predisposing factor for Hypertensive Nephrosclerosis

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The 677TT genotype of MTHFR, has been linked to an increased risk of CHD, an increased incidence of thrombotic episodes. Its relationship to HN has not yet been investigated. It has remained an enigma as to why some hypertensive patients develop nephrosclerosis and CRF, while others with similar degrees of hypertension do not. 217 patients with CRF and serum creatinines ranging from 2,0 mg% to ESRF, have had their C677T and A1298C polymorphisms of the MTHFR gene molecularly analysed. There were 141 males and 76 females with mean age of 63,3y. Their prevalence rates for the 677TT homozygous, double heterozygous 677CT/1298AC and 1298CC homozygous states, were compared to those of 92 controls using a chi-square test for equality of proportions with continuity correction. These patients were divided into 6 categories: PKD, DN, HN including patients with malignant hypertension, chronic GN, other renal diseases and unknown. The only significant differences in the prevalence rates of these polymorphisms were found between normals and the HN group, where the prevalence rate of the 677TT reached 50% (normal=13%,p=0), and that of the combined 1298AC/677CT was 39% (normal=31,5%), while none of these HN azotemic patients showed homozygosity for the 1298CC polymorphism (controls=16,3%). No significant differences in the prevalence rates of these polymorphisms were found when comparing normals and all of the 217 patients, and similarly no significant differences were found between normals and any of the 5 categories. The results suggest that hypertensive patients with the 677TT MTHFR genotype, may be predisposed to nephrosclerosis.

P0874. Intercellular adhesion molecule-1gene (ICAM-1)polymorphism: study of association with Multiple Sclerosis.

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Multiple sclerosis (MS), the common autoimmune demyelinating disease of young adults, is a chronic inflammatory disease of the central nervous system (CNS) characterized by primary demyelination with relative axonal sparing. Although the pathogenesis of MS is not fully understood, the role of genetic factors is firmly established. Several association studies of single genes have illustrated that genetic factors contribute to the increased risk to develop MS.

Intercellular adhesion molecule-1gene (ICAM-1) is membrane glycoprotein of the Ig superfamily consisting of five extracellular Ig-like domains, a transmembrane domain, and a short cytoplasmic tail. ICAM-1 plays an important role in inflammatory processes and immune responses. ICAM-1 is involved in the pathogenesis of multiple sclerosis (MS), whereas sequence variations in the its gene could potentially be responsible for the genetic susceptibility to MS. A common polymorphism of the ICAM-1 gene, A13.848G, has been recently reported.

In this case-control study, we evaluated the association between this polymorphism and MS by studying 202 patients and 217 age-matched controls.

All the patients and the controls were from the same ethnic background (Calabria region, Southern Italy).

No significant differences in genotype or allele frequencies were found between MS patients and controls.

We conclude that the ICAM-1 gene A13.848G, is not associated with the susceptibility to MS in our study.

P0875. Linkage analysis for AD using Amyloid Beta 42 levels shows evidence for a novel AD gene on chromosome 19

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For several families in which Alzheimer Disease (AD) segregates as an autosomal dominant trait, the genetic origin of disease remains unknown. The use of Amyloid beta 42 (Ab42) has led to the localisation of and AD related gene at chromosome 10. In order to identify genes implicated in the pathophysiology of AD, we performed a genome screen of AD in a Dutch family with mean onset of 64 years including eight patients. APP, PSEN1 and PSEN2 were excluded by direct sequencing. To gain additional phenotypic information, we measured Ab42 levels in 15 non-affected individuals still at risk for AD; through this way we identified 4 individuals with Ab42 levels over 205 pg/mL. We performed a NPL analysis, and defined our disease set in two ways. Our first analysis included only AD cases, and yielded NPL scores of 2.15 for marker D1S450, 2.12 for marker D7S2465 and 1.92 for marker D19S571. Second, an analysis of AD cases plus non-affected individuals with high Ab42 levels, yielded lower NPL scores for chromosomes 1 (0.20) and 7 (0.41), but increased the NPL score for chromosome 19 (3.09). Haplotype analyses showed a 30 cM region of chromosome 1 for which all AD patients were heterozygous. Also, all AD patients were compound heterozygous for haplotypes in chromosome 7 (~20 cM) or chromosome 19 (~40 cM). The chromosome 19 haplotype does not include the APOE gene. Combining the phenotypic data shows that the strongest evidence for the localisation of the AD gene is in chromosome 19.

P0876. Apolipiprotein B 3'Minisatellite-Polymorphisms in a romanian Population: correlation with total Cholesterol levels

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The apolipoproteins A, B and C play an important role in the metabolisms of plasma lipoproteins, lipids and in regulation of cholesterol. The apolipoprotein B-gene cluster is located in chromosome 2p2.23 and in the literature more than 25 different alleles (25-59 repeats) have been describes for this Apo B 3' Minisatellite. To search for a useful genetic marker for the hypercholesterolaemia in a Romanian population, the distribution of the alleles and their association on total plasma cholesterol level was investigated in 110 romanian individuals from Prahova Valley in the Carpatian Mountains. Genomic DNA was obtained from blood cells. DNA extraction followed the salting method (Miller et al., 1988, Nucleic Acids Research) and PCR amplification were done with specific primers for this minisatellite. PCR products were separated in polyacrylamide gels and visualized by silver staining. The distribution of all alleles in the Romanian sample is in Hardy-Weinberg equilibrium and show no significant differences to other European populations (like Italian, German, Hungrois, Greeces, Slowaks and Croatians) with an excess of the alleles 35 (27,40 %) and 37 (36,40%). We found also a statistical significant association between some allelic types (Repeats 32, 34 and 44) and high level of total plasma cholesterol concentrations (more than 180 mg/100 ml), as this group had nearly twice more level values than the other group. Therefore our results suggest that some of the apo B alleles may be a genetic component of genetical risk factors in hypercholesterilaemia.

P0877. Association study of SNPS in the genes G72 and DAAO with bipolar disorder

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Bipolar Affective Disorder I (BPI) affects ~1% of the general population. Two brain-expressed genes, G72 and DAAO, have been shown to be functional partners in the NMDAR regulation pathway

and to be associated with schizophrenia (Chumakov et al., 2002). Hattori et al., (2003) reported association between markers in G72 and BPI. Both genes are located in chromosomal regions showing evidence for linkage with the disorder: G72 on 13q33; DAAO on 12q24, making them good positional candidates as well.
We investigated five SNPs in *G72* (rs2391191, rs778294, rs954581, 326+4502C>A, 326+53G>C) and four SNPs in *DAAO* (rs3825251, rs2111902, rs3918346, rs3741775) in three samples: 170 BPI nuclear families of Bulgarian origin (BG), 116 BP families and 174 BPI unrelated cases and 170 controls of British origin (UK). All SNPs were genotyped by using the AMPLIFLUOR™ method.
Allele frequencies distribution did not reveal an association with BPI in ether sample. Haplotypes analysis for different combination of SNPs in both genes provided some positive results. Several two-, three and four-marker haplotypes in *DAAO* were associated with BPI (global p-values ranging from 0.0029 to 0.023) in the UK case-control sample. One three-marker haplotype was also significant in the UK trios sample: p=0.025. No haplotype was significantly associated in the Bulgarian sample.
Our results suggest that variants in the DAAO gene may be involved in the aetiology of BPI but we provide no support for the involvement of G72 in the aetiology of this disorder.

P0878. Genetic mapping of a *Ptch*-associated rhabdomyosarcoma susceptibility locus on mouse Chromosome 2

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Mutations in the *Patched* (*Ptch*) gene are responsible for various familial and sporadic cancers. *Ptch*$^{neo67/+}$ mice, in which exon 6 and 7 are deleted, show genetic background-dependent susceptibility to the development of muscle tumors resembling human rhabdomyosarcoma (RMS); BALB/c (BALB) is a susceptible strain whereas C57BL/6 (B6) shows resistance. A genome-wide linkage analysis was carried out using *Ptch*$^{neo67/+}$ mice produced from B6x(BALBxB6) backcrosses to identify loci involved in the control of RMS susceptibility. Quantitative trait locus mapping with the censored tumor latency time as the quantitative parameter was used to detect a RMS susceptibility modifier locus, *PARMS-M1* (*P*atched-*A*ssociated *R*MS *M*odifier 1), on chromosome 2 between D2Mit37 and D2Mit102 (LRS=10). A Kaplan-Meier survival curve revealed that mice with the B6/BALB genotype develop tumors more frequently and much faster (median latency time of 86 days) as compared to mice homozygous for the B6 allele (median latency time of 161.5 days (P=0.02)). Additional loci not reaching linkage significance were also detected for medulloblastoma resistance.

P0879. Genetic linkage study in the large autosomal dominant Columbian Family with essential tremor

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Essential tremor (ET) is the most common adult movement disorder with an age-specific prevalence ranging between 0.4% and 6.7% amongst people over 40 years. In this study we report of a genetic study of a large multigenerational (five-generation) Columbian family with autosomal dominant ET. DNA samples from 30 family members including 10 patients were available for genetic linkage analyses. All affected individuals have a similar clinical phenotype. A linkage analysis was carried out using a set of nine short tandem repeat (STR) markers from previously mapped loci of ET on chromosomes 3q13 and 2p22-25. Genotyping was performed on an automated DNA analyzer ABI 310. Two-point linkage analysis was performed with the LINKAGE package, version 5.1. Recombination frequencies between markers were calculated according to the UCSC map. For STR markers from chromosome 3q13, significantly negative LOD scores were obtained, that is why ETM1 locus of ET was excluded. Genetic linkage analysis with STR markers D2S2221, D2S320, D2S387, D2S2233, D2S262, D2S2168, D2S144, D2S174 and D2S131 at 2p22-p25 was performed and in the last four markers the most positive LOD scores was shown. A maximum two-point LOD score of 3.19 was obtained for D2S144 at recombination fraction θ = 0,04 . Genetic fine mapping is being performed now with other STR markers located between D2S131 and D2S144 in order to clarify whether the disease gene in this family is linked to ETM2 locus or ET represents a novel genetic entity.

STR- marker	LOD score (Z) at recombination fraction (θ)							Z_{max}	θ_{max}
	.00	.01	.05	.1	.2	.3	.4		
D2S131	- ∞	-3.24	-0.65	0.27	0.86	0.87	0.56	0.87	0.285
D2S2168	- ∞	2.15	2.60	2.57	2.16	1.56	0.82	2.62	0.057
D2S144	- ∞	2.74	3.16	3.08	2.57	1.85	0.98	3.19	0.060
D2S174	- ∞	1.85	2.30	2.27	1.86	1.26	0.55	2.31	0.058

P0880. Allele distribution model and simulation for genome-wide case-control analysis to search the trait loci of the multi-factor and incomplete penetrating disease.

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Case-control analysis with single-point SNP will be mainly used for large scale and high-density genome-wide analysis to search for the trait loci of complex diseases because of the high power and the ease to collect samples. However, because complex disease should include plural causes, the analysis for a trait locus should be disturbed by other trait loci. In each trait locus, because the case population includes the homozygotes of the non-disease-relevant allele, the probability of symptom of the homozygotes of non-disease-relevant allele must not be negligible and the genotype relative risk should be low. Therefore, case-control analysis for complex diseases should be more difficult than for single-cause diseases. The essential purpose of the case-control analysis is to search the marker loci with high linkage disequilibrium for trait loci. Hence, the statistics depending on only the linkage disequilibrium are ideal results. However, chi^2 statistics (and P values from them) by case-control analysis do not depend on only the linkage disequilibrium but also on several other parameters. The aims of this study are to test the effect of these parameters for the case-control analysis by simulation and to indicate appropriate values for effective analysis. By this simulation study, it is indicated that the low heterozygosity SNP marker loci are effective for the analysis requiring exact significance level because of multiple-testing and the analysis of the disease-relevant genes with low frequencies. In addition, two methods to increase the power, increase of sample population and cutback of non-relevant allele in case subjects, are introduced.

P0881. TNF-α and the risk profile of coronary atherosclerosis

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The proinflammatory cytokine TNF-α has been implicated in the pathogenesis of numerous complex diseases. Plasma level of TNF-α was associated with a variety of different risk factors, but only little is known about complex interactions. In this clinical study correlations were studied between plasma levels of circulating TNF-α protein (ELISA), mRNA expression in monocytes (RT-PCR) and genetic variants of TNF-α gene (SSCP) with several diseases, including obesity, athero-sclerosis, diabetes mellitus, hypertension and with risk factors such as age, gender, markers of inflammation, coagulation/ fibrinolysis balance, and lipid metabolism. In this study 194 clinically and biochemically well characterized patients were enrolled. In bivariate regression analyses significant positive associations between TNF-α protein level and obesity ($p<0.04$), age ($p<0.04$), body mass index ($p<0.02$), fibrinogen ($p<0.002$), plasminogen ($p<0.02$), and uric acid ($p<0.001$) were determined whereas HDL-

cholesterol (p<0.006) was shown to be negatively correlated. However, investigating confounding effects in a multivariate linear regression analysis, only age tuned out to be significantly associated with plasma levels of circulating TNF-α (p<0.05). The mRNA was not associated with any clinical or biochemical parameter investigated. Investigating the influence of genetic variants of TNF-α gene on transcriptional and translational level only one polymorphism (c.-238G>A) was shown to be associated with the mRNA but not with the protein.
Increased TNF-α protein levels, considered to be a risk marker of complex diseases, like atherosclerosis, were strongly associated with the age of the patients but not with classical risk factors, such as gender, smoking, blood pressure, diabetes mellitus or hyperlipidaemia, proven by multivariate analysis.

P0882. An association study of the cholesteryl ester transfer protein TaqI B and lipoprotein lipase S447X gene polymorphisms and ischaemic stroke in Greek patients

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Common ischaemic stroke is a multifactorial disease with a largely undefined genetic component. The involvement of genes affecting cholesterol homeostasis in the development of brain vascular disease, has prompted us to examine the putative association of two gene polymorphisms which reportedly affect plasma levels of HDL-cholesterol, namely cholesteryl ester transfer protein (*CETP*) TaqI B and lipoprotein lipase (*LPL*) S447X, in Greek stroke patients and control individuals.
Our study group consisted of 83 late onset ischaemic stroke patients (44 males, 39 females, mean age =75 years). The control group was age- and sex-matched and consisted of 73 individuals with no history of vascular disease.
No statistically significant differences were detected with respect to either the *CETP* TaqI B (genotype frequencies: patients, B1B1 37%, B1B2 46.9%, B2B2 16.1%; controls, B1B1 33.3%, B1B2 44.4%, B2B2 22.2%, *P*=0.610) or the *LPL* S447X polymorphism (patients, SS 78.3%, SX 20.5%, XX 1.2%; controls, SS 78.1%, SX 20.5%, XX 1.4%, *P*=0.990) when studied separately or in combination (interaction term not significant). An apparent trend towards an overrepresentation of the TaqI B2B2 genotype in the control group was found however (*P*=0.323), which was more prominent in those individuals that were non-carriers of the *LPL* 447X allele (*P*=0.225). We conclude that, even though an association between *CETP* TaqI B and/or *LPL* S447X and ischaemic stroke was not established in this study, the possibility that the TaqI B2B2 genotype confers a protective effect on a genotypically defined subgroup of the population, is worth examining in larger studies.

P0883. Influence of genes within inflammation pathways on type 2 diabetes

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Type 2 Diabetes mellitus (T2DM) accounts for about 90% of all diabetes cases worldwide and is a heterogeneous group of disorders caused by inherited and/or acquired deficiency in insulin production and/or insulin action. Chronic subclinical systemic inflammation leading to insulin resistance is hypothesized to play an important role in the development of T2DM. Therefore we analyzed 12 single nucleotide polymorphisms (SNPs) in 6 candidate genes within the inflammation pathways: *monocyte chemotactic protein 1* (*MCP1*), *macrophage migration inhibitory factor* (*MIF*), *monokine induced by gamma interferon* (*MIG*), *interleukin 13* (*IL-13*), *interleukin 18* (*IL-18*) and *interleukin 6 receptor* (*IL-6R*). Association of 12 selected SNPs with T2DM was tested among 236 probands suffering T2DM, 242 probands with impaired glucose tolerance (IGT) and 244 sex and age matched control subjects from KORA S2000, a German population based study. Genotyping of the SNPs was conducted using the MassARRAY system (Sequenom, San Diego, USA). All genotyped SNPs were in Hardy-Weinberg equilibrium. Statistical analysis revealed an association between one SNP in the promoter region of *IL-6R* and T2DM versus the KORA control group (p= 0.040). This result may give further evidence for an involvement of inflammatory genes in T2DM pathogenesis. Additional association studies and functional analyses are needed to clarify the role of IL-6R in the pathogenesis of the disease.

P0884. Association studies using high density SNP analysis in autism candidate genes

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Autism (MIM 209850) is a severe neurological disorder with a strong genetic background that affects four to ten individuals per 10000 births and persists throughout life. Symptoms include impairment in communication and social interactions as well as repetitive behaviour. The complex underlying mode of inheritance suggests involvement of several genetic factors.
Eight independant genome screens revealed strong evidence for linkage between chromosome 7q and autism and defined a 40 cM candidate region containing more than 900 genes. However to date no specific susceptibility genes have been detected.
To identify specific genes and gene variants playing a role in the etiology of autism, we genotyped a sample of 120 German trios fullfilling all diagnostic criteria, with or without language delay, using MALDI TOF MS technology for high throughput SNP genotyping.
For each trio we investigated 14 positional and functional candidate genes located in the 7q region. The criteria for candidate gene selection included involvement in brain development and brain metabolism.
We analysed 297 densely mapped, database-derived and partially validated single nucleotide polymorphisms (SNPs) located within or close to the exons of the genes of interest providing for a consistent coverage.
Analysis of these 106000 genotypes will allow for subsequent association and haplotype studies.

P0885. Association of estrogen receptor dinucleotide repeat polymorphism with osteopenia in very low birth weight (VLBW) infants

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Osteopenia is a metabolic bone disease which affects about 30 percent of VLBW infants. It has been reported that osteoporosis in adults is associated with polymorphisms of vitamin-D-receptor (VDR) gene, estrogen receptor (OER) gene and collagen 1α1 (COL1A1) gene. Based on these findings we investigated, whether such an association exists in VLBW infants.
Forty-five VLBW infants (24 female -21 male, mean gestational age: 29±2.4 weeks, birthweight: 1107±254 gm) were enrolled. At the age of 2, 3, 6, 12 month biochemical parameters for bone metabolism were measured. At the age of 2 and 6 months an X-ray of the chest and the wrist were taken. Blood sample was collected to identify the polymorphisms.
Results: Fifteen VLBW infants (33.3%) were diagnosed with osteopenia according to radiological signs, high levels of serum

alkaline phosphatase, osteocalcin, urinary pyridinium cross-links and calcium excretion. No difference was detected with regard to VDR and COL1A1 genes. However, a statistically significant difference in the distribution of the estrogen receptor gene polymorphism was found. Sixteen out of 30 infants without osteopenia and 1 out of 15 infants with osteopenia had 19 or 20 thymine-adenine repeat polymorphism (OR:0.06, 95%CI:0.00-0.54) and in 6 infants diagnosed with osteopenia and in 2 infants without osteopenia 18 repeat polymorphism was revealed (OR:9.33, 95%CI:1.29-103.96). **Conclusion:** Genetic variation at the OER locus appears to be associated with certain determinants for bone metabolism, 19 and 20 repeat polymorphisms play a protective role, 18 repeat polymorphism seems to be a risk factor in the development of osteopenia.

P0886. Parkin gene dosage analysis in late onset Parkinson's disease families

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Early onset (< 40 years) Parkinson's disease (EOPD) is an autosomal recessive neurodegenerative disorder characterized clinically by early onset of typical signs of parkinsonism. Pathological hallmark consists of selective loss of neurons in substantia nigra without Lewy bodies. The responsible gene was identified and designated *parkin*. The *parkin* gene, located on chromosome 6q25.2-27, contains 12 exons spanning 1.53 Mb and encodes an E2-dependent E3 ubiquitin protein ligase, that has a role in the proteasome-mediated degradation of target substrates. To date, a variety of mutations in the *parkin* gene have been reported, including exonic rearrangements (deletions and multiplications) and several missense mutations. The mutations are found predominantly in early-onset cases and the frequency decreased with increasing of age at onset (AAO).
For evaluating the role of the *parkin* gene in late onset Parkinson's disease (LOPD) cases, we performed a molecular screening of the 12 coding exons in 18 families originated from southern Italy, with probable autosomal recessive Parkinson's disease and an AAO of 45 years or older.
The conventional mutational analysis included polymerase chain reaction (PCR) and sequencing of the PCR products of the 12 exons on both strands. For identify any exon rearrangements we performed gene dosage analysis using real-time PCR 7900 HT-SDS (Perkin Elmer-Applied Biosystems).
We did not find any *parkin* mutations either by the conventional mutational analysis or with real-time PCR in 18 late-onset PD families. Our results suggest that *parkin* gene do not play an important role in developing LOPD.

P0887. Outward and inward directed aggressive behavior: association study of some dopamine- and serotonergic systems genes polymorphisms

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Several lines of evidence suggest common genetic mechanisms of both outward and inward directed aggressive behavior. It is known both serotonin and dopamine system dysfunction is associated with aggressiveness.
The aim of our study was to test a contribution of 9 polymorphisms of some candidate genes in aggressive behavior, namely - 5-HTTVNTR and 5-HTTLPR in the serotonin transporter (SLC6A4), A1438G in the serotonin 2A receptor (HTR2A), G861C in the serotonin 1B receptor (HTR1B), VNTR in the dopamine transporter (SLC6A3), TaqIA in the dopamine D2 receptor (DRD2), EcoRV in the monoamine oxidase A (MAOA), Val158Met in the catechol-O-methyltransferase (COMT), and A218C in the tryptophan hydroxylase (TPH) genes.
193 suicide attempters (males and females), 197 violent offenders (males) and 301 control subjects of the same ethnic background were typed for the above-mentioned gene variants using PCR technique.
We found a significant association of the COMT gene H/H genotype (suicide: OR= 2.28, 95%CI= 1.39-3.77; violent crime: OR= 1.84, 95%CI= 1.08-3.16) and the HTR1B gene G/G genotype (suicide: OR= 1.56, 95%CI= 1.04-2.35; violent crime: OR= 1.94, 95%CI= 1.19-3.19) with both types of aggressive behavior. Association of the TPH gene A allele (OR= 1.69, 95%CI= 1.08-2.63), 5-HTTVNTR of the SLC6A4 gene 12/10 genotype (OR= 1.86, 95%CI= 1.16-2.98) with suicidal behavior in females only, and of the DRD2 genotype A2/A2 (OR= 1.59, 95%CI= 1.04-2.41) with violent crimes were shown. However, there were no significant differences in genotype and allele frequencies distribution of any investigated polymorphisms between groups with outward and inward directed aggressive behavior.

P0888. Pro-opiomelanocortin (POMC) variants in morbidly obese Finnish children and adults

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Aims: The pro-opiomelanocortin (POMC) gene codes for several peptides involved in energy-balance regulation, including α -melanocyte stimulating hormone (α -MSH) and β -MSH. POMC mutations are a rare cause of severe early-onset obesity. Additionally, several variants of the gene have been associated with obesity-related phenotypes. The aim was to investigate the occurrence of POMC mutations and polymorphisms in children with severe early-onset obesity and in morbidly obese adults, and furthermore to relate their presence to metabolic parameters.
Methods: The three exons of the POMC gene and flanking regions were sequenced in 91 children with severe early-onset obesity (weight for height ≥ +70% before age 10, 42 females / 49 males). In addition, 250 morbidly obese adults (BMI ≥ 40 kg/m^2, 182 females / 68 males) and 191 healthy controls (95 females /96 males) are currently examined for genetic variants.
Results: Table 1. The POMC variants found in 91 severely obese children.

Variant		*Prevalence (n=91)*	
Nucleotide	*Amino acid*	*Heterozygous*	*Homozygous*
C7662T	S68S	11	-
7676/7677ins9bp	73/74insSSG	13	1
C7726T	L90L	1	-
C7965T	A169A	11	-
A8021G	E188G	2	-
C8246T	(5'UTR)	19	-
G8469C	(5'UTR)	1	-

Variants 73/74insSSG, E188G and G8469C were chosen for further determination of allele frequencies in morbidly obese adults and background population.
Conclusions: Several POMC gene variants, but no dominant obesity causing mutations were found among children with severe early-onset obesity. The possible relationship between different laboratory parameters and genotype is currently investigated.

P0889. Genes PARK1 and PARK2 analyses in Parkinson disease patients from the Volga-Ural region

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Parkinson disease is a neurodegenerative disorder, characterized by selective degeneration of dopaminergic neurons within the substantia nigra. The etiology of idiopathic PD is still undefined, but it is considered that PD is due to genetic susceptibility factors interacting with environmental exposures.
We studied 200 patients aged 48-83 years (mean 62 years) with PD from the Volga-Ural region. All patients showed at least two of the four principal signs of PD (resting tremor, rigidity, bradykinesia, postural-reflex impairment) and had no clinical features of any other

parkinsonian syndrome.
The 200 control subjects were collected according their age, ethnic origin, gender, area of residence. Controls demonstrated no significant signs of cognitive or neurological impairment.
Using SSCP analysis of all 12 exons of PARK2, followed by sequencing of shifted exons, we found Ser167Asn in the 4th exon and Asp394Asn in the 11th exon in 2% of PD patients. Such mutations were not found in controls. In introns 2 and 4 we detected +25 T->C transition and -25Adel respectively. Additionally, we found a novel heterozygous exon 12 deletion (Trp450Stop) in 2 unrelated patients of Russian origin. We tested for A53T and A30P mutations in PARK1, but no PD patients had these mutations. Our data show that PARK1 mutation is a very rare cause of PD in patients from the Volga-Ural region.
PARK2 sequence variants can result in affected proteins with loss of function and PARK2 mutations may cause Parkinson disease development.

P0890. Interaction between I/D polymorphism of *ACE* gene and C825T polymorphism of *GNB3* gene in type 1 diabetic nephropathy

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Vascular complications of diabetes are polygenic disorders. The I/D polymorphism of the *ACE* gene and C825T polymorphism of the *GNB3* gene have been implicated as independent risk factors for diabetic nephropathy in type 1 diabetes in some, but not all, studies. Angiotensin converting enzyme (ACE) catalyses the step that generates angiotensin II, and also inactivates bradikinin peptides, which play a key role in modulating vascular tone. The plasma ACE level is under genetic control and is strongly associated with the I/D polymorphism of the *ACE* gene. The 825T allele of *GNB3* is associated with enhanced G-protein signaling. The *ACE* I/D variant affects the hormones of the renin-angiotensin system, that activate G-protein-coupled receptors. The aim of this study was to investigate interaction of these genes in subjects with diabetic nephropathy.
The study consists of 74 patients with diabetic nephropathy and 92 controls without diabetic nephropathy matched to the patients by age, gender and diabetes duration. These subjects were characterized for *ACE* I/D polymorphism employing standard primers. The C825T polymorphism of *GNB3* gene was detected by the restriction fragment length polymorphism method after a polymerase chain reaction. Interaction between genotypes was estimated using logistic regression analysis. No significant interaction between genotypes at *GNB3* and genotypes at the *ACE* locus was observed with respect to diabetic nephropathy in type 1 diabetes. This finding indicates that these two genetic markers do not contribute synergistically to this vascular complication of diabetes.

P0891. Paraoxonase Dimorphisms: Independent Risk Factors for Ischemic Stroke

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Introduction- Ischemic Stroke (IS) is a heterogeneous disorder with multiple risk factors. HDL-associated serum paraoxonase (PON) enzyme prevents atherosclerosis by inhibiting oxidation of LDL. Though associated with cardiovascular disease, the role of PON in IS remains controversial. The aim of this study was to determine the association of *PON* gene polymorphisms with IS and to identify possible interactions with other vascular risk factors.
Methods- We analyzed 210 IS patients and 393 age- and gender-matched controls. We determined genotypes of 7 *PON* polymorphisms using polymerase chain reaction and restriction digestion assays. These included five *PON1* (-909 G/C, -162 A/G, -108 C/T, Q192R, L55M) and two *PON2* (148 A/G, 311 C/S) polymorphisms. We also determined serum PON1 activities by measuring rates of hydrolysis of both paraoxon and phenylacetate.
Results- Among the seven studied polymorphisms, two appeared strongly associated with IS risk (Table 1). Frequencies of -108T and 148A alleles were significantly increased in patients compared with controls (0.57 *vs.* 0.46, $P<10^{-3}$ and 0.49 *vs.* 0.42, $P<10^{-2}$, respectively). Besides, in the pooled sample of patients and controls, 909C alleles were associated with increased levels of phenylacetate activities (46.9 *vs.* 52.6 *vs.* 60.9 amongst GG, GC and CC, respectively, $P<10^{-3}$).
Conclusions- This study is the first to report independent associations of *PON* -108C >T and 148A>G genetic markers with increased risk of IS, and of 909G >C with phenylacetate activity. This would suggest that PON is implicated in the etiology of IS, and more extensive studies are needed to establish the causative mechanisms.
Association of the PON polymorphisms with Ischemic Stroke

PON Polymorphisms	Genotypic Association	Allelic Association
-108 C>T	p=0.004	p<0.001
148 A>G	p=0.022	p=0.007
Q192R, L55M, 909G/C, 162A/G,C311S.	NS	NS

P0892. Interleukin-1 Receptor Antagonist Gene Polymorphism and Multiple Sclerosis

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Multiple sclerosis (MS) is a multifactorial demyelinating disorder of the central nervous system connected with autoimmune reaction. The genes encoding various cytokines are logical candidates for MS susceptibilit. We investigated whether a polymorphism in the interleukin-1 receptor antagonist (IL-1 RA) gene is associated with both susceptibility to and clinical characteristics of MS.
Genotypes were determined from 64 patients with clinically definite MS and 118 healthy controls. All the subjects were unrelated, Russian, from Sverdlovsk region. Patient files were reviewed for disease type, initial symptoms, age at onset of disease, and rate of disease progression.
No significant differences in genotypes and allele frequencies were found between MS patients and healthy controls. Stratification for disease type (relapsing-remitting, primary progressive, or secondary progressive) did not provide significant differences between patients and controls. But we have detected an absence of allele IL-1RA*IV in controls, and a tendency of increase of IL-1RA*I/IV genotype (P=0.097) and IL-1RA*IV allele frequency (P=0.096) in patients MS with primary progressive type. In patients with disorders of co-ordination in initial symptoms we have detected a tendency of increase of IL-1RA*II/II genotype (25.0% Vs 7.62%, P=0.086) and significant increase of IL-1RA*II allele frequency (54.16% Vs 27.96%, P=0.001; OR=3.04), and decrease of IL-1RA*I/I genotype (8.33% Vs 49.15%, P=0.011; OR=0.09) and IL-1RA*I allele frequency (41.66% Vs 68.64%, P=0.01; OR=0.32) compared with controls. No associations were found with disease severity.
These results suggest that IL-1RA polymorphism is specifically associated with clinical characteristics of current disease.

P0893. A study of the hemostatic system genes and NO syntase genes contribution into stroke development

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Cerebrovascular pathology stands on the second place among the major mortality causes. One of the risk factors is genetic predisposition. In our study, the group of patients (N=108) was divided into ischemic (N=92) and hemorrhagic (N=16) stroke forms and in addition into light, intermediate and heavy forms, depending on the severity of the disease. The control group (N=100) consisted of the individuals not having symptoms of cardiovascular diseases. The allele frequencies were studied for genes *NOS3* (VNTR, C774T), *FV* (G1691A, A4070G), *FII* (G20210A), *GpIIIa* (T196C). Comparison of the allele frequencies was done using Chi-square criterion and Fischer exact test. There were no differences in allele frequencies of the polymorphisms in *FV*, *FII*, *GpIIIa*, *NOS3* (VNTR, C691T) between the patients and controls. Some effect of the C774T polymorphism

of *NOS3* gene was shown. The frequency of allele "T" was 25.5% in patients and 20.1% in controls (P=0.03). There was also difference in VNTR polymorphism in *NOS3* in different stroke types. The frequency of "A" allele was 21.7% in ischemic stroke group and 28% in hemorrhagic stroke group (P=0.03). Increase of the Leiden mutation (*FV* G1691A) and "T" allele of the C691T polymorphism in *NOS3* was shown in the group with heavy form of stroke, where their frequences were 9.4% and 21.9%, whereas in the controls they were 2.1% (P=0.02) and 12% (P=0.03) respectively.
The results suggest that *NOS3* polymorphism has some effect on the predisposition to stroke. The Leiden mutation may have prognostic importance for the severity of the disease.

P0894. Inherited thrombophilias (factor V Leiden mutation, factor II G20210A gene mutation and C677T MTHFR mutation) in Romanian women with unexplained pregnancy loss

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Introduction: Inherited thrombophilias such as factor V Leiden mutation, factor II G20210A gene mutation and the thermolabile variant C677T of the methylentetrahydropholate reductase (MTHFR) gene may be responsible for some cases of unexplained pregnancy loss.
Materials and methods:: This study was performed on 56 consecutive women referred for evaluation of thrombophilia because of pregnancy loss and on 42 women with at least one normal pregnancy and no history of pregnancy loss.
These three polymorphisms were investigated by polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP).
Results : The frequency of factor V Leiden mutation was greater among women with unexplained pregnancy loss (32.14%) than among control women (9.52%) (OR 4.5, 95%CI [1.39-14.54], p<0.01). In the same study the frequency of factor II G20210A gene mutation was greater among women with unexplained pregnancy loss (10.71%) than among control women (2.38%) but without statistical significance (OR 4.92, 95%CI [0.56-42.53], p=0.23). Concerning C677T MTHFR mutation we obtained the following results : the frequency of C677T MTHFR gene mutation was greater among women with unexplained pregnancy loss (67.86%) than among control women (14.29%) (OR 12.67, 95%CI [4.52-35.49], p<0.01).
Conclusions : Factor V Leiden and C677T MTHFR gene mutations but not factor II G20210A prothrombin gene mutation represent risk factors for pregnancy loss.

P0895. Association of FKBP1B and VDR gene polymorphisms in a large Tunisian family affected with thyroid autoimmune disorders

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The autoimmune thyroid diseases (AITDs) include two related mutifactorial disorders: Graves' disease (GD) and Hashimoto thyroiditis (HT). Based on the role of FKBP1B in the immunosuppressive pathway of FK 506 and the effect of vitamin D on endocrine system and its immunomodulatory properties, we analysed the role of the vitamin D receptor (VDR) and FKBP1B genes as candidate genes to AITDs susceptibility.
Sixty two patients belonging to a large Tunisian family affected with AITDs subdivided into 32 patients with GD and 30 patients with HT were genotyped for three VDR polymorphisms (Fok I in exon 2, Bsm I in intron 8 and Taq I in exon 9) and for (C/T) dimorphism located in 3' UTR region of FKBP1B gene. Data on VDR gene polymorphisms were analysed under three different clinical models corresponding to the affection status attributed to patients (GD, HT and both diseases combined).
Our results showed a significant association of allele C of 3'UTR FKBP1B gene using the Family-Based Association Test (FBAT) (p=0.018 under recessive mode). Concerning the VDR polymorphisms, FBAT analysis showed a high significant association of BsmI polymorphism (p =0.03 and p=0.015 under additive and recessive modes, respectively) for the combined model (GD+HT). This association showed a higher significance in the GD subgroup (p=0.02 under additive model). For HT subgroup, it is rather FokI polymorphism which showed the highest significance of association (p=0.024 under recessive mode). These results are suggestive of association in this family between AITDs and both FKBP1B and VDR genes.

P0896. Implications of Y-chromosome microdeletions and mutations on CFTR and Androgen Receptor genes to male infertility of testicular origin

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Spermatogenic deficiency can be only partially explained by acquired conditions identified by routine clinical evaluation. In order to highlight the potential contribution of genetic factors to the aetiology of testicular failure (TF), a comprehensive genetic analysis of Y-chromosome microdeletions, and a mutational screening of the AR and CFTR genes were performed with a thorough clinical evaluation in 83 TF patients, and compared with a fertile (F) control group (45 individuals for Y-chromosome and AR studies and 22 for CFTR analysis). Relevant clinical risk factors and abnormal karyotypes were identified in 33% of the TF population. STS-PCR analysis of the Y-chromosome showed the presence of microdeletions in 6% of cases. The CAG repeat length in exon 1 of AR gene in TF patients (mean±SEM=21.7±2.7) did not differ from that of F controls (21.5±3.2), nor did the range of repeat number (12-27 in TF patients and 14-29 in F controls). The mutational analysis of AR exons 4 to 8 was also performed, although no abnormal pattern was observed suggesting the absence of a determinant role of the AR gene in the aetiology of spermatogenic defects. Additionally, complete analysis of the CFTR gene coding region (27 exons) was performed. CFTR mutations were identified in 25.3% of cases and 18.2% of controls, however, no statistical conclusion could be raised due to the reduced number of controls analysed. Other risk factors were identified in some of the CFTR mutation carriers such as varicocele and cryptorchidism (6%) and Yq microdeletions (2.4%) suggesting TF as a multifactorial disease.

P0897. Cytogenetic study indicates that the amplification of MSRV pol sequence is significantly greater in patients with multiple sclerosis than in normal individuals.

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In the multiple sclerosis (MS), the demyelination process in the brain and spinal cord is observed. The bacterial/viral infections and genetic/immune factors in the etiology
of multiple sclerosis is postulated. The multiple sclerosis-related retrovirus (MSRV) is classified as a potential agent, which can lead to development of the disease.
The principal purpose of our cytogenetic studies was the assessment of MSRV pol sequence copy number in MS patients compared to normal individuals.
Interphase nuclei and extended chromatin fibers were prepared from peripheral blood of 16 patients with MS and 10 healthy individuals. In MS patients, the disease was clinically defined (the relapsing/remitting stage) and their age ranged from 20 to 57 years. The control group consisted of healthy individuals, aged 20-56 years.
The fluorescence in situ hybridization (FISH) with biotinylated PCR product was used to analyze of MSRV pol sequence copy number in the examined material. Detection of MSRV pol probe was carried out by reaction with avidin-fluorescein
and biotinylated anti-avidin.

MSRV pol sequences were found both in MS patients and in healthy persons.
Our results indicate that the copy number of MSRV pol sequence was significantly greater in MS patients than in normal individuals. In addition, the MSRV pol sequence exists as tandem repeats on various chromatin fibers (chromosomes). The increased number of MSRV pol sequence has been found on chromatin fibers of MS patients as compared to healthy controls. This finding suggests that MSRV sequence may play some role in the pathogenesis of MS.

P0898. Association between matrix metalloproteinase-1 promoter polymorphism G-1607GG and susceptibility to chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow obstruction that is not fully reversible. Disease results from an interaction between host and environmental factors, which vary in relative importance between affected individuals. Cigarette smoking is the major environmental risk factor for the development of COPD; however, not all smokers develop clinically significant COPD. The search for genetic risk factors for COPD represents a complex issue. Matrix metalloproteinase-1 (MMP1) is the most highly expressed interstitial collagenase degrading fibrillar collagens, the most abundant proteins in the human body. MMP1 expression is regulated by the upstream promoter sequences. A common polymorphic site, consisting of an insertion/deletion of a single guanine (G) nucleotide at position -1607, has been identified in the core recognition sequence of the binding sites for transcription factors that modify the level of MMP1 expression. Promoters containing the 2G allele display significantly higher transcriptional activity than 1G promoters.
Experimental Design: We genotyped for these 1G/2G polymorphism 160 COPD patients. A control population of 123 age- and sex-matched subjects was also genotyped for the same polymorphism.
Results: The proportion of 2G homozygotes was higher in the COPD group than in the controls (P = 0.014; odds ratio, 2.00; 95% confidence interval, 1.11– 4.16).
Conclusions: Genetic polymorphism in the promoter of MMP-1 gene may be associated with individual susceptibility to the development of COPD, because the MMP1 2G/2G genotype is predominantly found in patients with COPD. It is suggested that 2G/2G genotype may be less protective against smoking injury.

P0899. Genetic aspect of aminoglycoside induced ototoxicity: Role of slow acetylator phenotype and the point mutation A1555G in the gene 12S rRNA of the mitochondrial DNA

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Genetic factors which determine the side effects of medical therapy are to be the main object of personified medical investigation.
23 patients belonged to the european race with severe sensoryneural aminoglycoside induced hearing loss were examined. Screening of the point mutation A1555G in the gene 12S rRNA of the mitochondrial DNA was performed by Tono T.et al. method (2001).Presents of this mutation increases the sensitivity of hair cells to aminoglycoside induced ototoxic reactions. Also we evaluated the N-acetyltransferase activity by D.A.Evans`s method (1993) (determining the sulfadimizine) of all patients.
We found one patient out of 23 patients (4.3%) with the point mutation A1555G in mtDNA. At the mean time we found that the majority(78.3%)of this examined group had a low and extremely low N-acetyltransferase activity (less than 20%). Slow acetylation is to be the cause for earlier ototoxic complications (by the end of first week of aminoglycoside administration),in contrast,ototoxic reactions in rapid acetylators occurs in the case of prolonged uninterrupted aminoglycoside therapy (more than 1.5-2 months) which is characteristic for patients with tuberculosis and cystic fibrosis. According to these results, slow acetylation is the common cause for ototoxic side effects of antibiotics in european race. Moreover, point mutation А1555G of mtDNA was revealed for the first time among patients with aminoglycoside induced hearing loss in europion population of Russia. Thus, preliminary determination of the acetylator type and elucidation of maternal hereditary on deafness can be a method in screening the potential risk group for aminoglycoside induced hearing loss.

P0900. Gene Polymorphism of Methylenetetrahydrofolate Reductase (MTHFR, C677T), Apolipoprotein E, Angiotensin-Converting Enzyme (ACE, I/D) and Cardiovascular Disorders in Dialysis Patients

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Cardiovascular disorders is the main cause of death in dialysis patients. The purpose of the present study is to determine the relationship between molecular genetics and environmental factors in development of cardiovascular disorders in dialysis patients.
Eighty-six dialysis patients (42 women, 44 men) were included in our study. The mean patient age and mean hemodialysis duration were 49.0 ±12 years and 72 ±58.0 months, respectively. In addition, we investigated 320 healthy individuals. Testing was done on all patients to determine gene polymorphism of MTHFR (C677T), ApoE, ACE (I/D) and serum levels of total cholesterol (T-Cho), low-density lipoprotein (LDL-C), high-density cholesterol (HDL-C), triglyceride (TG), lipoprotein (a) (Lp[a]), ApoA1, ApoB, total homocysteine (Hcy), parathormone (iPTH), folate, albumin, haemoglobulin, calcium, phosphate and immunoglobulin.
The distribution of the polymorphisms of ACE and MTHFR in dialysis patients as a whole did not differ significantly from that of healthy controls. However, for patients with cardiovascular disorders the prevalence of the ApoE (ε3) allele was significantly lower ($\chi 2=4.48$, $p=0.036$). The plasma ApoB, TG and LDL-C concentration was significantly higher in patients with T (MTHFR) allele in combination with ε2, ε4 (ApoE) alleles. We also found that ACE D/D genotype is a significant predictor of the development of atherosclerosis damage of aorta (OR=3.75, $\chi 2=4.5$, $p=0.034$). We observed that the plasma homocysteine concentration was dependent on T (MTHFR) allele and hemodialysis duration.
In summary, our study shows that the development of cardiovascular disorders in dialysis patients depends on molecular genetics and environmental factors.

P0901. Angiotensin-Converting Enzyme (ACE) Insertion/Deletion Polymorphism and Environmental Factors in Development of Left Ventricle Hypertrophy in Haemodialysis Patients

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The goal of the study was to determine the predictors of left ventricle hypertrophy in uremic patients. 86 patients undergoing chronic bicarbonate haemodialysis (HD) were studied. Mean duration of HD was 72±58 months. Mean age was 49±12 yr. Male/female ratio - 1.04. Thickness of ventricular septum (VS) and posterior wall (PW) of the left ventricle were compared to I/D allele polymorphism of angiotensin converting enzyme (ACE), other echocardiographic parameters, age gender, arterial blood pressure (ABP-daily monitoring), body mass index, interdialytical weight gain, total cholesterol, low-density lipoprotein, high-density cholesterol, triglyceride, lipoprotein (a), ApoA1, ApoB, total homocysteine, parathormone, folate, haemoglobulin, calcium, phosphate and immunoglobulin. Univariate analysis shown that VS thickness was more than PW thickness in HD patients (1,33±0,3 vs. 1,21±0,24 sm, p=0,0001). Thickness of VS was higher in pts with DD-genotype in compare with I-allele carriers (1,49±0,3 vs 1,27±0,29 sm. P=0,005).
Thickness of VS was strongly correlated with male gender, diameter

of aorta, DD-genotype of ACE, aortic pressure gradient, diameter of right and left atrium, and ABP (all p<0,01). Stepwise multiple linear regression analysis have shown that significant predictors of VS hypertrophy are mean APB, diameter of aorta, aortic pressure gradient, aortic valve separation, and DD-genotype of ACE (Model R-square=0,47, p<0,0001). Only diastolic ABP was the predictor for PW hypertrophy (Model R-square=0,27, p=0,0005).
In summary, our study shows that in HD patients interaction of DD-genotype of ACE with environmental factors such as arterial hypertension and damage of aorta leads mainly to hypertrophy of VS as a part of left ventricle remodeling.

P0902. Genetic analysis of D543n and 3'-URT polymorphisms gene NRAMP1 in patients with pulmonary tuberculosis of Bashkortostan republic.

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Identifying the molecular basis of susceptibility to tuberculosis represents one of the most pressing problems of modern medical genetics. Sensitivity or resistance to infectious agents are known to be determined by genetic factors. One of the candidate genes, designated *NRAMP1* (natural resistance-associated macrophage protein), is associated with a susceptibility to tuberculosis.
We have studied the *D543N* and *3 ,-UTR* polymorphisms in *NRAMP1* in patients with pulmonary tuberculosis in the Republic of Bashkortostan.
Our sample comprised patients with pulmonary tuberculosis (108 persons) and 195 healthy inhabitants of Bashkortostan.
The *3 ,-UTR* polymorphism showed statistically significant differences between patients with pulmonary tuberculosis and healthy controls of Bashkortostan. The frequency of heterozygous TGTG/del was 5 times higher (15.89 %), than in the control group ($\chi 2 = 21.34$, OR=6.83). The D543N polymorphism did not show significant differences between the groups ($\chi 2 = 3.28$). Complex comparison of polymorphisms has shown, that patients with a DD/TGTG/del genotype have the highest risk of development of tuberculosis (OR=10.3).
We assume that changes in the structure of the NRAMP1 protein result in complete or partial loss of the ability to eliminate ions from the intraphagosomal space of macrophages, that favorably affects growth and duplication of mycobacteria and promotes a progression of tuberculosis.

P0903. The association analysis of some polymorphic DNA-loci with schizophrenia in different ethnic origin groups from Bashkortostan

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Some dopamine and serotonin system genes are suitable candidate genes to test for involvement in the pathogenesis of schizophrenia. VNTR- and 5-HTTLPR polymorphisms of the serotonin transporter gene (SLC6A4), VNTR-polymorphism of the dopamine transporter gene (SLC6A3), TaqIA RFLP of the dopamine D2 receptor gene (DRD2), Hsp92II RFLP of the catechol-o-methyltransferase gene (COMT), MspI RFLP of the serotonin 2A receptor gene (HTR2A) were analyzed using PCR method of DNA synthesis. 344 patients with schizophrenia from Bashkortostan (127 Russians, 109 Tatars and 108 Bashkirs) at the age of 15 - 74 were included in the analysis. The control group consisted of 423 persons (115 Russians, 168 Tatars and 140 Bashkirs). Our results indicate differences in genetic predispositions for schizophrenia in groups of different ethnic origins. Genotype HTR2A A/A (OR=2.21, 95%CI=0.97-5.04) and genotype 5-HTTLPR S/S (OR=2.04, 95%CI=0.98-4.27), genotype COMT H/H (OR =2.59, 95%CI=1.19-5.64) are risk factors for schizophrenia with continual type and genotype COMT H/H OR=3.15, 95%CI=1.41-7.09) is a risk factor for schizophrenia with episodic type in Tatars. Genotype SLC6A3 9/9 (OR=17.28, 95%CI=2.09-383.99) is a risk factor for paranoid schizophrenia with episodic type and genotype COMT H/H (OR=2.07, 95%CI=0.89-4.86) is a risk factor for paranoid schizophrenia with continual type in Russians. Genotype DRD2 A1/A2 (OR=2.13, 95%CI=1.02-4.47) and genotype COMT H/H (OR=2.74, 95%CI=1.22-6.23) are risk factors for schizophrenia with episodic type in Bashkirs. No significant differences in SLC6A4 gene VNTR locus were found between patients with schizophrenia and control group.

P0904. A genome wide scan for Attention Deficit/Hyperactivity Disorder: Loci in a German sample of Affected Sib Pairs

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Attention Deficit Hyperactivity Disorder (ADHD) is a common childhood-onset behavioral disease. About 5% of children are affected, and the disease persists in ~2% of affecteds until adulthood. It is characterized by hyperactivity, impulsivity, and inattention. ADHD is highly heritable and several studies have investigated a genetic component. The mode of transmission is not clear, but it is likely to be due to many genes each with a small effect. Several genome scans have been performed highlighting regions on chromosomes 5, 16, and 17. Obviously, abnormalities in dopaminergic neurotransmission are factors predisposing to ADHD. Patients respond to medications that inhibit the dopamine transporter. LD mapping studies at loci of single genes of the dopaminergic system have shown association with ADHD.
We have performed a whole genome scan with 101 families comprising 156 affected sib pairs. All patients were form Germany and fulfilled the DSM-IV criteria.
We have genotyped 409 STR markers for the scan and data have been used to perform statistical analyses. Using Genehunter, several regions of interest where detected. The most impressing peak was attained on chromosome 5q near the SLC6A3 gene polymorphism, previously described to be associated with ADHD. This and other putative gene regions where saturated with markers and data will be presented. In addition, other candidate gene regions have been tested.

P0905. Analysis of candidate genes for autism in the linkage region on 7q

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Autism is the prototypical pervasive developmental disorder characterized by social and language deficiencies and a restricted range of stereotyped repetitive behaviours with an onset in early childhood and persisting throughout life. Twin and family studies suggest a strong genetic etiology of autism and several linkage studies show evidence for a number of susceptibility loci. In order to identify predisposing genes for autism currently 317 patients from 257 families have been collected throughout Germany and Austria available for candidate gene screening, while 165 complete trios are available for association studies. In addition, IMGSAC has collected to date 290 families with more than one affected child or relative. Based on the results of the IMGSAC genome screen in 152 affected sibling pairs (ASP) indicating principal loci on chromosomes 2, 7, 16 and 17, further analysis of the expanded IMGSAC family collection including 219 ASPs provided continued support for linkage on chromosome 7q, generating a multipoint MLS of 2.44 between markers D7S530 and D7S640 at ~ 133 cM. Candidate genes with a function in brain or brain development within 7q21-q33 are being systematically screened for autism relevant mutations or variants

with DHPLC and sequencing followed by association studies with known or newly identified SNPs. To date several gene variants or associations have been identified in the German and/or IMGSAC sample with a possible role in the etiology of autism. However, replication in other independent samples is warranted, in order to clarify the role of these genes in autism.

P0906. Evidence for association of NOS3 gene polymorphism with earlier progression to end stage renal disease in a cohort of Hellens from Greece and Cyprus

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Nitric oxide (NO) is thought to be an important factor in the deterioration of renal function. A variable number tandem 27-bp repeat in intron 4 of the endothelial cell nitric oxide synthase (NOS3) gene has been found to be associated with the plasma levels of NO metabolites. Two alleles are of varied frequencies in different populations (a and b). The shorter allele, a, has been associated in Japanese populations with the progression of renal disease. Here we investigated this hypothesis by studying the role of this polymorphism in a Hellenic population of patients with end-stage renal disease (ESRD). We analysed the genotypes of 361 ESRD patients and 295 healthy Hellens from Greece and Cyprus. The data in the two populations were analyzed by chi-square and Fisher's exact test. The frequencies of the three genotypes of NOS3-4 polymorphism in the Hellenic population of Greece and Cyprus are similar to those observed in other Caucasian populations. Moreover, our results from three patient groups, ADPKD, DM and non-DM, showed that the frequency of aa and ab genotypes in the patient populations were not significantly different from those observed in the control group. This work indicates that NOS3-4 polymorphism does not show any association with the development of ESRD in this studied European population. However, examination of the data as regards progression to ESRD following clinical diagnosis of ADPKD, provided evidence of statistical difference (P=0.048, before Bonferroni correction), with faster progression in the group of ADPKD patients who carried allele a.

P0907. Autozygosity mapping and assessment of homozygosity by SNP-chip

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The development of a silicon chip, such as the Affymetrix 10K Xba 131, bearing sufficient oligo-nucleotides to analyse 10,913 autosomal single nucleotide polymorphisms (SNPs) presents a new method to seek autosomal recessive loci. We have devised a spreadsheet that analyses the raw SNP allele output of the SNP-chip. The spreadsheet results are assessed by probability of occurrence. Analysis of the SNP-chip suggests that the output is equivalent to 3cM spaced microsatellite markers for the detection of homozygous segments, but much better for heterozygous segments.
Comparing the methodology with polymorphic microsatellite markers, SNP-chips would be expected to be quicker and cheaper, use less DNA and detect smaller regions, however haplotypes cannot be easily derived. In two of the 11 families we have performed microsatellite autozygosity mapping linkage would not have been found using SNPs; one locus being sub-telomeric and one being <3cM, both were eventually detected by visual inspection of haplotype. For both methodologies coverage is often poor at sub-telomeric regions.
Using this approach we have also analysed individuals born of first cousins parents. The amount of their genome that was homozygous (and hence probably autozygous) varied from 3-14%.

P0908. Polymorphism of PLAT and PAI genes in development of preeclampsia

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BACKGROUND: Preeclampsia is known as one of the most serious and relatively common complications of pregnancy. Endothelial dysfunction is thought to be the final common pathway leading to the clinical syndrome of preeclampsia. An important role in the regulation of fibrinolytic activity and blood pressure is played by plasminogen activator inhibitor type 1 (PAI1) and tissue plasminogen (PLAT) activator. Polymorphisms of corresponding genes (4G/5G of the PAI1 promoter and Alu insertion-deletion (I/D) of the PLAT) are associated with elevated or decreased activity of the relevant gene products.
METHODS: By means of PCR assay we analyzed the frequency and distribution of the 4G/5G polymorphism of the PAI1 gene and the I/D polymorphism of the PLAT gene in the DNA samples of 114 women with preeclampsia history (patients) and of 79 healthy female (controls).
RESULTS: The frequency of 4G/4G genotype of the PAI1 gene in preeclampsia patients (45%) was significantly higher compared this one in controls (30%, p<0.05). No differences were registered between genotypes and alleles distribution in I/D polymorphism of the PLAT gene for both groups.
CONCLUSION: The presence of 4G/4G genotype of the PAI1 gene, which is associated with higher activity of PAI1 and thus with increased blood pressure, could be suspected as a risk factor for development of preeclampsia.

P0909. Prevalence of Angiotensin Converting Enzyme (ACE) gene insertion-deletion polymorphism in Sickle Cell Disease patients

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Genetic factors interact with sickle cell mutation to manifest the phenotypic heterogeneity of sickle cell disease. The insertion-deletion (I/D) polymorphism of the ACE gene has been identified as a genetic risk factor for many diseases including those with vascular involvement. The objective of this study was to investigate an association between ACE genotypes and the onset/course of sickle cell disease in Kuwaiti Arabs. Blood samples were collected from SCD patients (n, 113; 226 chromosomes) and Hb-AA controls (n, 48; 96 chromosomes). Total genomic DNA was extracted by a standard method and ACE genotypes were determined using polymerase chain reaction (PCR) followed by analysis of PCR products by agarose gel electrophoresis. Allele frequencies were determined by gene counting. The I/D polymorphism of ACE gene is bi-allelic and therefore three genotypes are expected. The incidence of homozygous deletion (DD) genotype in Kuwaiti SCD patients was 65% compared to 52% in controls. The heterozygous ID genotype was detected in 27% patients compared to 46% of controls. The homozygous insertion (II) genotype was detected in 9% patients compared to 2% of controls. The differences in the prevalence of DD and II genotype in Kuwaiti SCD patients and controls are not significant and therefore indicate that this polymorphism does not constitute a risk factor in the onset or heterogeneity of SCD. However, its role in SCD patients with specific complications e.g. renal failure still needs to be determined.

P0910. A genomewide scan for uric acid in two different collections of Utah families

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Background: There is strong evidence that uric acid is an effective antioxidant. It is the most abundant scavenger of free radicals in humans and its effect is substantially greater than that of vitamin C. On the other hand, several prospective studies found high uric acid levels to be associated with total mortality and cardiovascular disease. It was hypothesized that higher uric acid levels try to compensate an increased oxidative stress long before atherosclerosis becomes visible and that it interacts with other antioxidants to protect against developing atherosclerosis.
Hypothesis: There exist genes which influence uric acid levels as suggested by segregation analysis.

Methods: We performed a genome-wide search for regions which harbour genes responsible for increased uric acid levels in two different data sets from Utah. The first one consisted of 48 pedigrees ascertained because of two or more first-degree relatives with early cardiovascular disease, the second one consisted of 98 large pedigrees ascertained for early coronary heart disease deaths, early stroke deaths, and early onset of hypertension. We performed nonparametric multipoint linkage analysis.
Results: The most interesting region was found on chromosome 6 with a LOD score of 3.6 in the second data set and 1.1 in the first set. A further locus which suggested evidence for linkage was observed on chromosome 9 with a LOD score of 2.5 in the first data set which was not confirmed in the second data set.
Conclusions: There is evidence that high uric acid levels are linked to a locus on chromosome 6.

P0911. Predictive value of the HLA-DRB1 shared epitope for radiographic damage in rheumatoid arthritis depends on the individual patient risk profile

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Objective: Genetic factors influence disease progression, but also predispose to non-genetic predictors of prognosis. Thus, the additional value of genetic information in the prediction of prognosis may depend on the patient's risk profile. Our aim was to evaluate the additional value of HLA-DRB1 alleles encoding the rheumatoid arthritis (RA) shared epitope (SE) to the prediction of radiographic damage in RA conditional on non-genetic predictors in individual patients.
Methods: Demographic characteristics, baseline clinical characteristics and HLA-DRB1 genotypes were available for 180 Caucasian women with RA. Univariate analyses were performed to select predictors of radiographic damage. The additional value of the SE for the prediction of radiographic damage was determined by a newly developed method estimating the likelihood ratio (LR) of SE status conditional on other predictors of radiographic damage. These LRs were used to calculate the sensitivity and specificity of the SE testing for each woman in the study.
Results: Overall, the LR of SE presence (LR+) was 1.4, the LR of SE absence (LR-) 0.4, the odds ratio (OR) 4.0, sensitivity 0.85 and specificity 0.40. The SE was a significant predictor of radiographic damage (multivariate OR 4.6 [95% CI 1.6, 13.2]). Taking into account the risk profiles of individual women, the LR+ varied from 1.0 to 2.3, the LR- from 0.2 to 0.5, the sensitivity from 0.60 to 1.00 and the specificity from 0.00 to 0.78.
Conclusion: The additional value of SE testing for radiographic damage in individual women with RA varies according to the risk profile of the patient.

P0912. Association of MTHFR C677T polymorphism and inflammatory markers, a risk factor for CHD; the ATTICA Study

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Prospective studies have identified many markers of systemic inflammation that are powerful predictors of future cardiovascular events in patients with coronary artery disease (CAD) and in apparently healthy subjects. A common polymorphism in the methyl enetetrahydrofolate reductase (MTHFR) gene C677T, is one cause leading to increased homocysteine levels and is considered to be a genetic risk factor for cardiovascular disease.
This study was designed to determine the relationship between the levels of inflammation markers and MTHFR genotype among subjects of the ATTICA study. We investigated demographic, lifestyle, clinical, biochemical and genetic information from 322 men (46 ± 13 years) and 252 women (45 ± 14 years), without any clinical evidence of cardiovascular disease, from the ATTICA study. Among other characteristics we measured total plasma homocysteine levels. The MTHFR genotypes distribution was: homozygous wild type (CC), 41%; heterozygous (CT), 48%; and homozygous mutant (TT) genotype, 11%. Homocysteine, C-reactive protein, fibrinogen, white blood cell counts, and amyloid levels were higher in TT compared to CC genotypes, in both males and females, even after controlling for different confounders through multi-way ANCOVA. In conclusion, this study demonstrates that these observations have implications for risk factor management in the primary prevention of cardiovascular disease in healthy individuals.

P0913. Periodic catatonia: systematic analysis of candidate genes in a schizophrenia locus on chromosome 15q15

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In a genome-wide linkage study on 12 extended multiplex pedigrees segregating for periodic catatonia (MIM 605419) we recently identified a major disease locus on chromosome 15q15, and replicated the chromosomal locus in an independent set of four pedigrees.
The disorder is characterized by qualitative hyperkinetic and akinetic psychomotor disturbances through acute psychotic episodes, and debilitating symptoms in the long term with psychomotor weakness, grimacing facial movements, and apathy.
Linkage and haplotype analysis in three exceptionally large pedigrees linked to chromosome 15q15 revealed an 11 cM critical region between marker D15S1042 and D15S659. In our efforts in identifying the disease gene we performed linkage disequilibrium (LD) mapping and haplotype analyses with a set of low informative microsatellite markers and SNPs in multiplex pedigrees and parent-offspring trios. Concurrently, we carried out systematic mutation scan of candidate genes annotated in this critical region. Among the brain expressed genes we have so far analysed by automated sequencing the coding region including the exon-intron boundaries as well as large fragments of the 5'- and 3'-UTR regions of several candidate genes in individuals from the linked pedigrees and controls. No disease causing mutation was identified in ARHV, VPS18, CHP, ITPKA, KIAA0252, KIAA1403, KIAA0770, TYRO3, and SNAP23. Systematic linkage disequilibrium mapping and family-based and case-control association studies in the gene loci is ongoing.

P0914. Interaction of MTHFR C677T polymorphism and Mediterranean Diet: effect on homocysteine levels in healthy adults; the ATTICA Study

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Plasma homocysteine is an independent risk factor for coronary heart disease and may be influenced by dietary, as well as genetic factors. In the presented study we evaluated the relationship among the adoption of Mediterranean diet and Methylenetetrahydrofolate Reductase (MTHFR) C677T polymorphism, on homocysteine levels, in healthy adults from the ATTICA study.
We studied demographic, lifestyle, clinical, biochemical and genetic information from 322 men (46 ± 13 years) and 252 women (45 ± 14 years), without any clinical evidence of cardiovascular disease. Among other characteristics we measured total plasma homocysteine levels, the distribution of MTHFR genotype and adherence to the Mediterranean diet (as assessed a diet score).
The distribution of MTHFR genotypes was: homozygous wild type (CC), 41%; heterozygous (CT), 48%; and homozygous mutant (TT) genotype, 11%. Homocysteine levels were higher in TT compared to CC and CT (15.8 ± 9 vs. 11.3 ± 8 vs. 10.8 ± 9 μmol/L, $p < 0.001$). Adoption of the Mediterranean diet was not associated with homocysteine levels ($p = 0.89$). However, stratified analysis revealed that adherence to Mediterranean diet reduced homocysteine levels in TT and CT individuals (Beta = -0.41, $p = 0.002$ and Beta = -0.14, $p = 0.025$, respectively), but not in CC (Beta = -0.08, $p = 0.18$), after controlling for several potential confounders. The observed effect of MTHFR C677T gene – diet interaction on homocysteine levels may provide a pathophysiological explanation by which Mediterranean diet

may influence coronary risk in people with increased homocysteine levels.

P0915. Increased prevalence of glycoprotein IIa/IIIb Leu33Pro polymorphism in term infants with grade I intracranial haemorrhage

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Platelet glycoprotein IIb/IIIa PLA2 allele (Leu33Pro), which has already been suggested to have a role in the development of idiopathic thrombocythaemia, and factor V G1691A Leiden mutation were determined in samples pooled from 109 appropriate for gestational age neonates with grade I intraventricular haemorrhage (IVH). The control group is composed of 118 IVH free infants.
The neonates with IVH were subgrouped into 55 mature and 54 premature neonate and the controls were separated into subgroups of 58 term and 60 preterm infants. The PLA2 allele frequency was 16.4% in the group of full term infants with grade I IVH, while it was 9.5% in the relevant controls ($p<0.005$). There was no difference within the PLA allele frequencies comparing the IVH affected (8.34%) and unaffected (9.2%) premature infants. Contrary to these findings, factor V Leiden allele frequency was increased in the subgroup of premature infants with grade I IVH as compared with the appropriate premature controls (9.25% vs 3.34%, respectively, $p<0.005$). The prevalence rates of factor V Leiden allele showed no difference compared to the IVH affected and unaffected full term infants (2.72% vs 2.59%, respectively). These data suggest that both PLA2 allele and factor V Leiden mutation can have significance in development of the events of IVH, but the susceptibility is maturation dependent.

P0916. C677T Methylentetrahydrofolate reductase polymorphism and risk of premature coronary heart disease in men in a Russian population

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The methylentetrahydrofolate reductase (MTHFR) plays a key role in metabolism of plasma homocysteine. The C677T MTHFR gene polymorphism is accompanied by reduction of enzyme activity and increased homocysteine level.
In this study we investigated the association of the coronary artery disease (CAD) risk in young men group with MTHFR gene polymorphism.
333 men have been examined: 157 men suffering miocardial infarction (MI) under the age of 45 and 176 healthy men. Identification of C677T MTHFR gene polymorphism was carried out by means of polymerase chain reaction. Lipid spectrum and intima-media complex thickness (IMT) of common carotid artery were measured in all patients by enzyme method and the doppler method, respectively.
We have found that the frequency of the T-allele of MTHFR gene in patients was higher than in control group (0.33 and 0.27, respectively, $p=0.03$). Among CAD patients IMT was higher than in healthy individuals (0.96±0.03 mm and 0.79±0.01 mm, respectively; $p=0.0001$). The triglyceride, common cholesterol, low-density lipoprotein levels were significantly higher, and the high-density lipoprotein levels were significantly lower in the patient group.
Risk of MI was 20% higher in patients with T-allele of MTHFR gene than in CC patients. Thus, the presence of T-allele of MTHFR gene is independent risk factor for premature CAD.

P0917. Analysis of candidate genes for autism on chromosome 2q.

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Autism is a severe neurodevelopmental disorder, likely to arise on the basis of a complex genetic predisposition. In order to identify susceptibility genes, the IMGSAC performed a whole genome screen for linkage in affected sib-pair families and identified the strongest linkage on chromosome 2q31-q33 (MLS of 3.74 at D2S2188 in 152 sib-pairs). Supporting evidence for the presence of an autism susceptibility locus on chromosome 2q is provided by the convergence of linkage findings from independent genome screen studies. We are systematically screening functional candidate genes mapping in the region of linkage, to identify variants that may contribute to the aetiology of autism. To date, we have analysed fourteen genes, nine of which have already been reported in a publication (Bacchelli et al, 2003). Here we present the results obtained from the mutation screening and association studies of five new candidates: NCKAP1, CED-6, FRZ-B, INPP1 and KIAA1604. All coding regions and putative functional sequences have been screened by DHPLC and sequencing in 32 to 48 affected individuals from IMGSAC families that are contributing to the linkage peak on 2q. DNA variants identified through the screening have been tested for association with autism by case-control and/or TDT studies in the whole IMGSAC family sample.
No evidence was found that any of the above candidate genes strongly contributes to autism susceptibility. However a SNP in INPP1 gene provided a nominally significant result in the TDT test, which warrants further investigation.

P0918. Effect of Spirapril therapy on endothelium functional status in patients with premature miocardial infarction with different genotypes of Angiotensin Converting Enzyme (ACE)

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The aim of our study was to investigate the effect of spirapril therapy on endothelium functional status in patients with miocardial infarction with different ACE genotypes.
60 patients with coronary artery disease (CAD) were examined. All of them had history of miocardial infarction. The mean age of the patients was 52.0±1.0 years, and the mean age of the begining of CAD was 42.1±1.0 years.
Testing was done on all patients to determine I/D polymorphism of ACE gene. Endothelium-dependent vasodilatation (EDV) of brachial artery was determined using reactive hyperemy test. Serum level of aldosterone and activity of renine were measured by radioimmune method.
Distribution of I and D alleles were 0.55 and 0.45, respectively. EDV was decreased in all patients (4.8±1.0%). Furthermore, EDV in DD patients was reliably lower than in I allele carriers (3.69±0.12% and 9.75±1.7%, respectively; $p=0.03$). There were no significant differences in activity of renine and serum level of aldosterone in patients with different ACE genotypes. Spirapril therapy led to reliable rise of EDV of brachial artery ($p=0.02$). The greatest increase was observed in DD subjects.Thus, we established endothelial disfunction in all CAD patients, and it was maximal in DD patients. Spirapril therapy improved endothelium functional status in all of them, especially in DD subjects.

P0919. Characterization of a candidate gene involved in cardiac hypertrophy

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In order to identify candidate genes contributing to the development of cardiac hypertrophy in human, we have screened differentially expressed genes in the heart of spontaneously hypertensive rats (SHR) which provides a well known animal model to investigate hypertension and heart failure.
Based on a substractive hybridization system we identified several downregulated and upregulated genes in cardiac tissue of SHR. Real Time PCR analysis of one of them, clone 65,with yet unknown function revealed a significantly increased transcription level in 12 weeks old SHR compared to normotensive WKY rats as control.

Further experiments like EST-clustering and in silico analysis lead to the identification of a 3.4 kb rat gene on chromosome 13q11 with a putative ORF of 1211 bp. The homologous murine and human genes could be localized on MMU 1E2.3 and HSA 2q14.1, respectively. Linkage analysis of HSA 2q14 revealed this region to be involved in hypertension based on a genomic wide scan in Scandinavian sib-pairs, empasizing clone 65 to be a good candidate gene. Therefore, further characterization of clone 65 may lead to identification of a new locus associated with initiation or progression of cardiac hypertrophy

P0920. A role genes of detoxification processes in pathogenesis of sporadic amyotrophic lateral sclerosis in Russian population.

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The main process, which may be involved in ALS pathogenesis, is oxidative stress. The important role in prevention of oxidative damage plays detoxification process of xenobiotics. This process can be can be divided into two parts: phase I - the biological activation of xenobiotics and phase II - detoxification of phase I products. The major participants of phase I are cytochromes P-450 (CYP2E1, CYP2D6) and phase II are glutathion-S-transferases (GSTM1, GSTT1, GSTP1) and N-acetiltransferses (NAT2). To investigate a role of genes of detoxification system in development of SALS, we study polymorphisms in these genes in 62 patients with SALS in Moscow and controls from Russia. Analysis of insertion (I) polymorphism was revealed statistical distinction in frequency of II genotype between patients (11,3%) and controls (2,5%) (χ^2=10,71; P<0,05). Whereas, the analysis of genotypes for the CYP2D6*4 alleles has not found out significant distinction for CYP2D6*4 homozygous between patients (7,7%) and control (4,8%)(χ^2=0,62; P>0,5). Also, we have found no difference between different genotype variants for GSTM1, GSTT1 and GSTP1 loci in our samples. The comparative analysis for F/S_1,S_2,S_3 polymorphism of NAT2 gene (F - fast allele and S_1,S_2,S_3 - slow alleles) has revealed statistical distinction in distribution of allele frequencies between patients and controls (χ^2=10,64; P=0,01). We conclude that CYP2E1 gene and NAT2 gene, probably, can be involved in development of ALS in Russian population.

P0921. Hemochromatosis H63D allele is associated with severe oligozoospermia

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Recent studies suggest an increased incidence of genetic causes related to male infertility. In addition to the genes on Y chromosome, there is an increasing evidence for existence of genes on autosomal chromosomes that are implicated in spermatogenesis. Hemochromatosis (HFE) is an autosomal recessive disorder characterized by iron overload leading to hepatic cirrhosis, diabetes mellitus, hypogonadism and cardiomyopathy, if untreated. HFE gene is located in 6p21.3 region and recently a candidate gene for human male infertility was reported in this region. The aim of this study was to compare the frequencies of HFE mutations in infertile patients to that of fertile males (proven fathers). A total of 153 infertile patients and a control group of 145 proven fathers were studied by PCR/RFLP analysis for the presence of HFE C282Y and H63D mutations. No C282Y mutation was found among the two studied groups. The frequency of H63D mutation was higher among infertile patients (15.7%) than among controls (12.4%), but the difference was not statistically significant (p=0.251). However, when the frequency of H63D mutation was analysed in different groups of infertile patients according to their sperm counts, a significantly higher frequency was detected among patients with sperm count of <1x10^6/ml (24.2%, p=0.016) compared to the fertile males. These data suggest that HFE H63D allele is associated with severe oligozoospermia. HFE H63D might act as a modifier allele or it may be a marker for a linked gene implicated in spermatogenesis.

P0922. A novel family with X-linked Mental Retardation, Primary Ciliary Dyskinesia and Macrocephaly, linked to Xp22.32 - Xp21.3

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We report on a large three-generation family with mental retardation associated with primary ciliary dyskinesia (PCD). The 10-year-old index case had delayed motor development, severe mental retardation (IQ20), and macrocephaly. Recurrent respiratory infections led to the diagnosis of PCD at the age of 8 years. Chromosome analysis, MRI and metabolic screening were normal. There was no deafness or retinitis pigmentosa. 8 other affected males died because of bronchopulmonary infections before the age of 5 years. All were mentally retarded, and in some, hydrocephalus was suspected because of macrocephaly. In one boy, postaxial hexadactyly was described. None of the affected had situs inversus. 5 obligate female carriers were clinically inconspicuous.
We performed pairwise and multipoint genetic linkage analysis using 69 SNPs as markers, with an average interval of 3-5 cM. Tight linkage to DXS8019 was found with a maximum LOD score of 2,49, and the gene defect could be mapped to a 25 cM wide Xp22.32 - Xp21.3 interval. X-linked inheritance is rather uncommon for PCD and - to our knowledge - has not been described before in combination with severe mental retardation and macrocephaly. In one other family with mental retardation, hydrocephalus and ciliary dyskinesia, linkage to the X-chromosome was eventually excluded (Al-Shroof et al. 2001). Thus, we seem to have defined a new XLMR syndrome associated with macrocephaly and PCD. Mutation screening of candidate genes from the relevant linkage interval is in progress.

P0923. The IL10 -1082 AA genotype is significantly associated to the risk of neonatal respiratory distress syndrome (RDS)

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RDS is a lung disorder that primarily affects premature infants and causes increasing difficulty in breathing. The disease is caused by a structural immaturity of the lungs and immaturity of the pulmonary surfactant metabolism, resulting in surfactant deficiency and dysfunction. Apart from these factors there is increasing evidence that inflammation and clotting contribute to the progression of this disease. Genetic factors related to the inflammation may influence immune cells activation and cytokine release. 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 IL10 -1082 G/A in 22 in patients with neonatal RDS and in 109 normal controls. We observed a significant association between IL10 genotype and risk of RDS. Compared with AA homozygotes, the ORs of RDS cases for AG heterozygotes and GG homozygotes were 0,24 (95% confidence interval 0,08-0,73) and 0,59 (95% confidence interval, 0,17-2,10) respectively (P=0.032). It demonstrated that SNP allele -1082 G is associated with higher IL10 production respect to G/A heterozygous and A/A homozygous. In fact, the -1082A carriers (ie IL10 low producers) have been shown to be more likely to develop inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases. Our results suggest that differential production of IL-10 due to genetic variants might be a factor in the development of RDS.
Genotype distribution of IL10 in controls and patients [XSquare (2df)=6,90 P=0,032

Genotype	Controls n (%)	Cases RDS n (%)	OR (95% confidence interval)
AA	35 (32,1)	13 (59,1)	1 (refernce group)
GA	56 (51,4)	5 (22,7)	0,24 (0,08-0,73)
GG	18 (16,5)	4 (18,2)	0,59 (0,17-2,10)

P0924. Methylenetetrahydrofolate dehydrogenase G1958A polymorphism is a genetic determinant of NTD risk for Italian mothers

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Neural Tube Defects (NTDs) have a well-established genetic basis, although specific genetic predisposing factors had not been identified. Genetic variants of enzymes involved with folate pathway might be expected to have impact on NTD risk. Given its key role in folate metabolism, the methylenetetrahydrofolate dehydrogenase (MTHFD1) could represent an attractive candidate in NTD etiology. Recently, a polymorphism, G1958A, in the MTHFD1 gene was suggested as maternal genetic risk factor for NTD in the Irish population. We examined the impact of the MTHFD1 G1958A polymorphism on NTD risk in the Italian population by a case-control and family-based studies. The study population consisted of 95 NTD children, 42 mothers, 40 fathers, and 145 controls individuals. The presence of MTHFD1 G1958GA, MTHFR A1298C and RFC-1 A80AG polymorphisms was investigated by PCR-RFLP methods. We found no increased risk for MTHFD1 mutant genotypes of the children and fathers. Significant risk estimates resulted for the mutant homozygous 1958AA genotype of the mothers (OR=3.05; P=0.046). This maternal effect was confirmed by TDT analysis that showed no preferential transmission of 1958A allele to the affected children from informative parents. Since our previous study has shown that the MTHFR A1298C and RFC-1 A80G polymorphisms are genetic determinant of NTD risk for Italian mothers, gene-gene interactions were examined. We found a significant interaction between the MTHFD1 G1958A polimorphism and both MTHFR A1298C (4.46-fold increased risk) and RFC-1 A80G (9.14-fold increased risk) mutant alleles of the mothers. We conclude that MTHFD1 G1958A polymorphism is a maternal risk factor for NTDs.

P0925. ‚Association of 5.1 allele of the MIC-A gene with insulin-dependent diabetes mellitus in children with recently determined diabetes‘

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Insulin-dependent diabetes mellitus (IDDM) is autoimmune disorder of multifactorial etiology with a strong genetic component. The frequency of IDDM in North-West region of Russia is 1-3:1000 newborn. IDDM primarily develops due to selective autoimmune destruction of the insulin-producing pancreatic beta cell, which leads to severe insulin deficiency. The recent studies have been demonstrated that polymorphism of the MICA gene (major histocompatibility complex class 1 chain-related genes) is associated with susceptibility to type 1 diabetes.

A total of 52 children with recently determined diabetes, 48 initially unaffected siblings with the level of cytoplasmatic islet cell antibodies titer above 28 Juvenile Diabetes Foundation Units and 97 unrelated non-diabetic peoples from North-West region of Russia were analyzed for the exon 5 polymorphism of MICA gene by PCR and DNA heteroduplex analysis. The group of children with recently determined diabetes included children with (21 patients) and without (31 patients) diabetic relatives.

The frequencies of alleles 4, 5, 5.1, 6, 9 of MICA gene were 7.1%, 19%, 42,9%, 9.5%, 21.4% in children with recently determined diabetes with diabetic relatives, 15.5%, 9%, 37.8%, 21.1%, 16.6% in unaffected siblings and 9.5%, 15%, 29.4%, 19%, 27.1% in the control group accordingly. Thus we discovered an increasing frequency of allele MIC-A 5.1 in unaffected siblings in comparison with population ($p<0,05$) and in IDDM patients with complicated inheritance in comparison with unaffected siblings ($p<0,05$). We suggest that the testing of MIC-A 5.1 allele may be recommended for predisposition analysis to IDDM in unaffected siblings from North-West region of Russia.

P0926. Glutatione S-transferase P1 (GSTP1) polymorphism in patients with chronic placental insufficiency (CPI).

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Background: Placental insufficiency is a process leading to progressive deterioration in placental function and a decrease in transplacental transfer of oxygen and nutrients to the fetus. As known, the product of the GSTP1 gene is one of the important xenobiotic metabolizing enzyme in reproductive tract and placenta.We have studied the polymorphisms of the glutatione S-transferase P1 gene in patients with chronical placental insufficiency (CPI).

Methods: Polymerase-chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were applied for genotyping of GSTP1 polymorphisms in exon 5 (Ile105Val) and exon 6 (Ala114Val) in 31 DNA from placenta samples with CPI and 76 control samples.

Results: No differences were registered between genotypes and allele distribution of the Ile105Val polymorphism of the GSTP1 gene for both groups. An increased frequency of the Ala114Val allele was observed for the CPI group compared to the control group (21% versus 7% p >0,001). Carriers of the114Val allele were registered in 35,5% of the CPI group compared to 13,9% of the control group (p >0,001). The relative risk for CPI when having homozygous or heterozygous Ala114Val of GSTP1 gene was estimated by an odds ratio of 3.4 (95% CI 1.3-8.7).

Conclusions: It may be suggested that GSTP1gene variants, such as AlaVal114, could be a genetic risk factor for placental insufficiency.

P0927. Homocysteine levels and MTHFR C677T genotypes in patients with myocardial infarction

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Hyperhomocysteinemia is a possible risk factor of coronary artery disease. The C677T mutation in methylentetrahydrofolate reductase (MTHFR) gene is one of the causes of an elevated homocysteine plasma concentration and probably one of the atherosclerotic risk factors.

AIM : We assessed the association between homocysteine levels and MTHFR 677 genotypes in young patients with myocardial infarction (MI).

METHODS : Our group involved 43 patients with acute MI, 41 of them age </= 45y and two female older patients. The MTHFR genotype was analyzed using PCR amplification and digestion with restrictive endonuclease Hinf I. Total homocysteine sera concentration was measured using HPLC with fluorescence detection.

RESULTS : Among 43 patients with MI, 55.81% had C/C homozygous genotype, 32.56% had C/T heterozygous genotype and the remaining 11.63% had T/T homozygous genotype. The frequencies of alleles were 0.72 (C) and 0,28 (T). We obtained significant difference in frequency of T/T genotype in MI patients, compared with control group (T/T=6.9%). Mean homocysteine levels in our patients were: 11.93mcmol/l, 10.58mcmol/l and 9.68mcmol/l for C/C, C/T, and T/T group, respectively. There was no significant correlation between homocysteine levels and MTHFR677 genotypes.

CONCLUSION: Our results showed association of MTHFR677 T/T genotype and MI in young MI patients, but did not show genotype and homocysteine levels correlation. The determination of MTHFR677 genotype could be important in assessment of the risk of coronary disease in people age under 45y.

P0928. Susceptibility to Alzheimer disease: Genetic groupings and age at onset

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The very richness of the genetic underpinnings of Alzheimer disease may contribute to the difficulty in consistently identifying any one

susceptibility gene. To remedy this situation we employed a version of latent class analysis abbreviated as GoM to identify susceptibility groups from a roster of 50 candidate loci. There were 508 AD patients of widely varying age and 343 at-risk often middle-aged subjects. The five model-based groups representing the data were numbered I to V in order of increasing age: APOE44+ early-onset AD (I) was rich in inflammatory signaling markers and genes that modulate amyloid-beta production and degradation. APOE-unrelated AD (II) had a more specific and shorter list of pertinent loci. Both shared a more general set of predisposing factors. Unaffected (III) lacked risk genotypes. In addition, a SCD genotype was common, missing from the affected groups: This signals a predisposing process for all sorts of AD. APOE4+ late-onset AD (IV) was perversely low in other risk factors. Very late onset APOE33+ AD (V) carried a genotype for a histone acetyltransferase not found for the other groups implying a role for altered gene transcription. Using this statistical genetic approach chromosome 10q becomes readable carrying multiple relevant loci. Each group is defined by probabilities of carrying the genotypes. This model-based approach defined genetic susceptibility groups that implied processes and likely onset age. The membership scores for individuals in each group are potentially a tool to define risk for individuals.
Note: The first two authors contributed equally to the work.

P0929. Systematic association studies of the familial hemiplegic migraine gene ATP1A2 in migraine with aura

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The *ATP1A2* gene encodes the alpha2 subunit of a Na+/K+ pump. Recently is has been found that missense mutations in *ATP1A2* are responsible for familial hemiplegic migraine type 2 (FHM2), a rare monogenic form of migraine with aura (MA). In order to determine whether *ATP1A2* is also involved in the molecular pathogenesis of complex inherited MA, we performed systematic case-control association studies in 268 MA cases and 234 control individuals. By directly sequencing all 23 coding exons and adjacent intron regions of 40 MA patients, sixteen polymorphisms (12 SNPs, 3 small indels, 1 microsatellite marker) were identified. The sequencing results were used to estimate seven common *ATP1A2* haplotypes (with a frequency >5%) covering about 97% of total haplotype diversity for this region. Subsequently, 6 haplotype-tagging SNPs/polymorphisms were genotyped in 94 individuals with a family history of MA, in 173 individuals with sporadic MA and in a gender-matched control sample. A haplotype analysis was performed with the program COCAPHASE 2.3. No significant differences in the *ATP1A2* haplotype distribution could be detected between MA patients (or patient subgroups) and the control group. In a single-marker analysis the allele and genotype frequencies of *ATP1A2* polymorphisms between cases and controls were compared. Neither the 6 ht-SNPs nor a single allele of the microsatellite marker were significantly associated with MA. In summary, we found no evidence for a contribution of *ATP1A2* to the pathogenesis of complex inherited migraine with aura.

P0930. Statistical mapping power of single consanguine pedigrees segregating a recessive disease

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In every day clinical practice sometimes consanguine families are detected apparently segregating a so far unknown recessive disease. „What linkage power has this family?“ is the important question preceding further research. The probability of achieving a linkage Lodscore of greater than three can be estimated by wellknown simulation approaches. We evaluated the mapping power of selected real pedigrees under certain conditions. The power depends on the family structure, the availability of DNA, the disease allele frequency, the marker distance from the postulated gene locus and the marker characteristics. In consanguine families additionally the number of generations to the common ancestor play an important role. In our largest family the connecting ancestor of the patients lived six generations ago. Under standard assumptions for marker and disease the maximal reachable Lodscore is 3.8 but, surprisingly, the probability of achieving a Lodscore greater than three is only a few percent. This is a striking example for the possible difference between maximum and average Lodscore. But if we assume that a very rare haplotype can be found in affecteds on both chromosome copies and heterozygous in parents and unaffected sibs the power is very high. The more generations the common ancestor is apart the smaller the expected mean length of the shared interval among affecteds will be. If the comon ancestor of the patients lived more than 6 generations ago the shared segment may well be smaller than 3 cM. In such situations denser scans will rather lead to success.

P0931. The dystrobrevin-binding protein 1 (DTNBP1, dysbindin) gene in schizophrenia: a follow-up study in case-control samples of German, Swedish, and Polish origin

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We have recently performed an association analysis with SNPs in the gene for dystrobrevin-binding protein 1 (DTNBP1), or dysbindin, which has strongly been suggested as a positional candidate gene for schizophrenia, in three schizophrenia case-control samples of German, Polish, and Swedish descent. We identified significant evidence for association in the Swedish, but not in the German and Polish sample. The results in the Swedish sample became even more significant after separately analyzing those cases with a positive family history of schizophrenia. This suggested that genetic variation in the dysbindin gene could be particularly involved in the development of schizophrenia in cases with a familial loading of the disease (Van Den Bogaert et al., Am J Hum Genet 73:1438-1443, 2003). None of the associated SNPs appeared to be the risk-conferring variant itself, and the functional variant still awaits identification. We have now analyzed three additional SNPs in our samples comprising 850 schizophrenia patients and 670 controls, among them a recently identified functional SNP that was shown to have an effect on the expression level of the dysbindin mRNA. Single-marker and haplotype analyses are currently underway and results will be presented.

P0932. Minisatellite UPS29 is unstable in Parkinson’s disease patients.

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Increasing data evidence in favour of connection between minisatellites instability and onset of severe human diseases. Thus it is actual to search and characterize new human minisatellites both in norm and under pathologies. Conducted computer analysis of not enough studied GC-rich minisatellite UPS29 demonstrated its enrichment with some recombination motifs and is localized inside introne 13-14 of gene CENTB5 (1p36.33), which is alternatively spliced. Three loci associated with Parkinson disease are in the region 1pter-1p36.33. Sequencing of PCR product of UPS29 of DNA isolated from Parkinson patients showed rearrangements inside this minisatellite. These results point to a possibility that UPS29 instability could be one of etiological factor of this disease. (The work was supported by grants of RFBR 01-04-49634 and of President RF MK-2182.2003.04)

P0933. HFE gene mutations in autoimmune hepatitis-frequency and effects on ferritin level

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Objective: to determine frequency of C282Y and H63D mutations in patients with autoimmune hepatitis and to evaluate the effect of these mutations on ferritin level
Methods: we investigated the frequency of these mutations in 66 patients and 120 age- and sex-matched healthy controls. DNA extraction with salting-out method was performed on blood samples and afterwards, mutation detection based on PCR-RFLP method was done. C282Y and H63D mutations were sought in all subjects by digestion of PCR products with RsaI and BclI restriction endonucleases, respectively.
Results: There was no C282Y mutation in either patients or controls.14 (21.2%) patients and 26(21.7%) controls were heterozygote for H63D mutation, and two (3%) patients and two (1.7%) controls were homozygote for it. The H63D allele frequency was 13.6 and 12.5 in patients and controls, respectively (p=0.828). We did not detect any significant difference in ferritin level among patients with H63D mutation and the ones without it.
Conclusion: There is no marked difference in the frequency of H63D mutation between patients with autoimmune hepatitis and healthy controls. Besides we did not detect any C282Y mutation in our patients. These results also suggest that there is not any association between HFE gene mutations and ferritin level in autoimmune hepatitis.

P0934. No evidence for a genetic association of the XBP1 promoter polymorphism (-116C/G) with bipolar affective disorder in a large, collaborative study

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A recent study reported evidence that the XBP1 gene on chromosome 22q, which encodes a pivotal protein within the endoplasmic reticulum stress response pathway, contributes to susceptibility to bipolar affective disorder (BPAD). A functional polymorphism (-116C?G) in the promoter region, which changes the consensus motif for an XBP1 binding site, was significantly associated with BPAD in 197 Japanese probands with BPAD and 451 controls, as well as in 88 parent-offspring trios of (mainly) European-American descent. Given the potential impact of this finding, we attempted to replicate the association in a large collaborative study combining XBP1 (-116C?G) genotype data from family-based (n=586 trios; including the 88 trios used in the original report) as well as case-control samples (about 1,200 cases and 1,700 controls) of BPAD, all of European and European-American descent. Our samples were 6 times larger than those used in the original report and exceeded a power of 99% to detect an effect of the magnitude reported there. The results of our study do not provide confirmatory evidence for an influence of XBP1 (-116C?G) in the development of BPAD. We discuss possible reasons for failure to replicate the reported association. In a further step, we extended our analysis to unipolar depression and schizophrenia. This was based on the hypothesis that common genetic factors may be present in BPAD, unipolar depression and schizophrenia. Association analysis with these phenotypes was also negative.

P0935. MTHFR gene polymorphism as stroke risk factor in young patients

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Hyperhomocysteinemia is an established, independent risk factor in coronary, cerebral and peripheral vascular disease. The 5, 10 methylenetetrahydrofolate reductase (MTHFR) gene polymorphism C677T has been shown to result in increased total homocysteine concentrations caused by a decreased enzyme activity.
The aim of this study was to determine C677T genotype in stroke patients and investigate a possible association among the T677T genotype and homocysteine levels. C677T polymorphism was analyzed by PCR amplification of specific fragment of genomic DNA followed by Hinf I digestion. Homocysteine levels were determined by fluoropolarizationimmunoassay.
Group comprised 25 patients, age rang 21 to 52, with stroke.
We have found that genotype was wild type CC in 10 (40, 0 %), heterozygous CT 11 in (44, 0 %) and homozygous mutant TT in 4 (16.0%) patients. Hyperlipidaemia was found in 7 (28%) and mild hypertension in 5 (20%) patients. Sixteen (64%) patients had elevated homocysteine levels (>10.3mmol/L). Patients with MTHFR T677T genotype were younger than the others (t=2.635, p=0.025) and had highest homocysteine levels (t=0.324, p=0.048).
Although it is small group, our results indicate that such relation might exist, namely, that MTHFR T677T genotype is associated with younger age and more several clinical presentations.

P0936. The sequence variant of CARD15/NOD2 gene in Polish patients with Crohn's disease

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Crohn disease (CD) belongs to chronic inflammatory diseases of the gastrointestinal tract with unknown etiology. Genes of predisposition were localized on chromosomes 3, 7, 12 and 16 with the most significant locus on pericentromeric region of chromosome 16 (IBD1). Molecular analysis involved 3 exons of *CARD15/NOD2* gene located in this region. 114 persons affected with CD were examined. Sequencing showed 866A>G substitution, leading to Asp289Ser in NBD domain of NOD2 protein. We confirmed also presence of frequent Pro268Ser variant characteristic for CD patients in Caucasians (C - 0,61, T - 0,39). In control group allele T was less frequent (0,16). No changes were detected in exon 9. Analysis of exon 11 identified 3020insC, causing frame shift (1007fs) resulting in protein lacking 33 amino acids. We observed coexistence of variant Pro268Ser and 1007fs. In 10 cases 1007fs was present along with homozygous 268Ser. In two patients with homozygous 268Ser also homozygous 1007fs variant was found. In one CD family affected daughter showed homozygous presence of two frequent variants 802C>T (Pro268Ser) and 3020insC (1007fs). Homozygous variant 802C>T appeared in brother and father of affected girl as well, whereas mother was heterozygous. The second variant - 3020insC was homozygous only in patient. We conclude that the presence of Pro268Ser variant even in homozygote status is not univocally associated with the morbidity of CD. We confirm observations that 50% CD patients carry at least one mutation and hypothesis that a complete damage of the NOD2-signaling pathway may be necessary for development of the disease.

P0937. Association of SNP haplotypes in DRD3 gene with Parkinson's disease among Indians

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Parkinson's disease is a progressive neurodegenerative disorder characterized by bradykinesia, resting tremor, muscular rigidity, postural instability, afflicting 1-2% of the population above 55 years of age. The role of genetic factors in the etiology of PD is unambiguous

and at present, a total of 4 genes (Alpha synuclein, Parkin, UCHL1 and DJ1) and 7 chromosomal loci have been reported in ADPD and ARJP cases. But the mutations in these genes accounts for a very small fraction of PD cases mostly the familial ones and does not explain the vast majority of sporadic PD cases. Therefore association studies with SNPs in candidate genes from the dopaminergic pathway are one of the major approaches to understand the genetic etiology of idiopathic PD cases.
DRD3 is a D2 like receptor and an important receptor in dopamine mediated signaling. It has been implicated in neurodegenerative and neuropsychiatric illness. We have investigated association of six SNPs across the DRD3 gene with PD using a case-control approach (600 IPD patients and 450 age and sex matched controls). Out of six SNPs two were found to be monomorphic in 100 controls and 100 cases and therefore were not analyzed further. Of the remaining SNPs analyzed, no significant association was observed with PD. However, a significant association (p=0.01) was observed with the SNP haplotypes constructed with these markers using the Phase 2.0.2 software in our considerably large sample size. This is the one of few reports in which SNP haplotypic association has been observed instead of allelic associations.

P0938. Polymorphism 109T>G and 570A>T of *BMP2* gene and their association with osteoporosis in Polish patients

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Osteoporosis is a common disease characterized by decrease in bone mineral density (BMD) and microarchitectural deterioration of bone structure leading to higher susceptibility to fractures. Development of osteoporosis is multifactorial process in which environmental and genetic factors play an important role. Recent studies have indicated that majority (up to 80%) of variability in bone mass and density is genetically determined. Molecular genetic basis of osteoporosis remains difficult to define because bone mass, a major determinant of osteoporosis fracture risk, is quantitative trait, influenced by interaction between many genes and environmental factors. To date several candidate genes have been analyzed in relation to osteoporosis in many populations. Recently, linkage analysis in Iceland families showed LOD score of about 5.0 to chromosome 20p12.3 and a follow-up association analysis showed association to some polymorphisms in bone morphogenetic protein 2 gene (*BMP2*). We studied association of two *BMP2* gene polymorphisms (ref. NM_001200): c.109T>G (p.Ser37Ala) and c.570A>T (p.Arg190Ser) with osteoporosis, in group of 200 postmenopausal women representing Polish population.

P0939. Relationship between Hormone Replacement Therapy (HTR) and Sp1 polymorphism in post-menapousal cases with osteoporosis

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Osteoporosis is characterized by increased risk of bone fractures, and related with polymorphisms in various genes, including the genes determining the bone mineral density (BMD). Most of the studies were held on the G-T polymorphism in the Sp1 binding region in the first intron of the Col1A1 gene. In this study, Sp1 polymorphism was investigated by PCR-RFLP in 100 non-smoking patients with osteoporosis who have not received hormone replacement therapy for at least 3 years after menapouse, and in randomly chosen 100 healthy controls. All patients have received a conjugated 0.625mg estrogen/2.5mg medroxyprogesterone therapy. The BMDs were detected at lower lumbar region and femur neck in the beginning of the therapy and 18 months after, and the effect of HRT on BMD in cases with or without Sp1 polymorphism was investigated. In controls, 84% of the individuals had an SS genotype and 16% had an Ss genotype. SS genotype was detected in 76% of the cases with postmenapousal osteoporosis, whereas 24% had an Ss genotype. Upon comparison of the measurements done in the beginning of the therapy process, cases with Ss genotype was found to have lower BMDs compared to those with SS genotype. In the measurements done through 18 months, increase in the BMD was lower in cases with Ss genotype than that in cases with SS genotype. These results suggest that additional supportive therapy modalities besides HRT will be useful to provide an increase in the BMD and for preventing fractures in cases with osteoporosis with Ss genotype.

P0940. MTHFR C677T polymorphism and homocysteinemia in patients with Parkinson's disease

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Hyperhomocysteinemia (Hhcy) is a known risk factor for vascular diseases and neural tube defects, but recent studies analyze its role in the development and progression of neurodegenerative disorders, including Parkinson's disease (PD). The folic acid is an important cofactor in homocysteine (Hcy) metabolism, and methylenetetrah ydrofolate reductase (MTHFR) gene polymorphism C677T results in increased Hcy level by a decreased enzyme activity. In addition, in PD patients drug levodopa may increase Hcy level by influence on folate metabolism. Our study analyzed relationship between Hcy level, levodopa therapy and MTHFR 677 genotype in PD patients. We studied a group of 100 PD patients: 81 levodopa-treated and 19 levodopa-nontreated, and 38 control persons. In all patients complete neurological and neuropsychological examinations were performed. Plasma Hcy levels were determined by HPLC, and MTHFR C677T polymorphism was detected by PCR/RFLPs method. The results showed significantly increased Hcy level in parkinsonian patients compared with controls (levodopa-treated: 17.23±6.26; levodopa-nontreated: 17.52± 8.01 and control: 12.76±4.04 mmol/L, $p<0.001$). The frequencies of MTHFR 677 genotypes in treated, nontreated and controls were: CC: 45.7%, 26.3% and 34.2%, resp., CT: 42%, 52.6% and 50%, resp., and TT: 12.3%, 21.1% and 15.8%, resp. In all three groups TT genotype was associated with significantly higher Hcy level. We confirmed presence of Hhcy in PD, and association of Hhcy with MTHFR 677 genotype, but not with levodopa therapy. The Hcy measurement and MTHFR genotypisation are important in both levodopa-treted and nontreated patients, and folate/vitamine supplementation could prevent vascular or other PD complication.

P0941. Identification DelA3314 in mitochondrial ND1 gene in diabetic type 2 patients

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MtDNA has 16.5 kb that carries 37 genes, including 2 RNAs, 22 tRNAs, and 13 polypeptide subunits of enzymes involved in oxidative phosphorylation. There is a variant of mtDNA mutations which is A3243G tRNA(leu).
While high level (90%) of the A3243G mutation is associated with MELAS, a low level (50%) of this mutation is related to diabetes mellitus type 2 with or without deafness. A 5kb mtDNA deletion has been also reported in the patient's with diabetes mellitus type 2. We assessed the frequency of the A3243G and 5kb mtDNA deletion in Iranian diabetes mellitus type 2. DNA was extracted from blood of 140 diabetic type 2 patients. Insulin rate of the patients were also tested. PCR-RFLP and SSCP methods were used to detect the A3243G or other mutations in the mitochondrial tRNA(leu) gene. Standard and multiplex PCR were used to detect to 5kb deletion in patient's mtDNA. We could not identify any deletion or A3243G point mutation in our cases. SSCP results showed a new pattern of PCR product in 7 patients. Sequencing was done by 3700 ABI capillary system. An "A" nucleotide deletion in 3314 position was detected in

mitochondrial ND I gene. So far this deletion has not been reported.

P0942. IL-4 receptor alpha chain polymorphism and atopic dermatitis

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Atopic dermatitis and other atopic diseases have a strong genetic predisposition. A major role in the pathogenesis of atopic dermatitis plays the IL-4 system, especially the IL-4 receptor alpha chain. Mutations in this subunit of the IL-4 receptor have been reported to be more frequent in patients with atopic dermatitis than in healthy controls. However, the association apparently differs among ethnic groups. Therefore we screened a number of patients of the Salzburg region by complete sequencing of the IL-4 receptor gene. Subjects with atopic dermatitis were identified on the basis of clinical histories, positive RAST and elevated total IgE level.
Eight amino acid substitutions have been found: one substitution in the extracellular domain and the remaining 7 in the intracellular domain. Six probands showed one or more mutations in the IL-4 receptor alpha chain (6 the Q576R polymorphism, 2 the C431R, 1 the I75V and S436L). The number of mutations did correlate with higher levels of total IgE, whereas patients with low IgE levels tended to have no mutations. This is in contrast to the concept of intrinsic atopic dermatitis, which is supposed to have a stronger genetic background. We have shown that a high percentage (55%) of patients with atopic dermatitis shows polymorphisms in the IL-4 receptor alpha chain. Especially the Q576R allele, is very common (6/11) among Salzburg population. However, we could not find an association with lowered total IgE concentration for this polymorphism, as described previously, because 5/6 showed a total IgE of more than 1000 kU/l.

P0943. eNOS gene polymorphism (Glu298Asp) and stroke in Serbian population

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Background: The role of eNOS in normal physiology suggest that it could be a potential candidate gene for stroke. Reduced eNOS activity could mediate an increased stroke risk through hypertension or independent from hypertension through abnormal vasomotor responses, promoting atherogenesis, or increased platelet adhesion and aggregation. A common missense variant of the eNOS gene (Glu298Asp) has been reported to be a risk factor for coronary artery disease and essential hypertension.
Aim: The aim of this study was to determine the association if any between eNOS Glu298Asp gene polymorphism and stroke in Serbian population.
Methods: We studied 56 patients with stroke and 100 healthy controls. The presence of the polymorphism Glu298Asp was determined by polymerase chain reaction and digestion with *Mbo I* restriction enzyme.
Results: We found no significant difference in genotypes frequency distribution between patients with stroke and healthy controls. Frequencies for eNOS Glu298Glu, Glu298Asp, Asp298Asp genotypes in patients were: 44.64%, 44.64%, and 10.71% and in controls: 47.92%, 38.54%, and 13.54%, respectively. Allele frequencies were not significantly different between patients and controls.
Conclusion: According to our results eNOS gene polymorphism (Glu298Asp) is not associated with stroke in our population. Further studies with large number of patients and other candidate genes is still needed to confirm these results.

P0944. Genotype distribution and allele frequencies of the apolipoprotein E polymorphism gene in young men with mild essential hypertension in St.Petersburg, Russia.

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The aim of our study was to investigate the apoE gene polymorphism in young men essential with essential hypertension (EH), a control group and children as a population sample of St Petersburg.
The inclusion criteria were EH with systolic (S) blood pressure (BP) which fell in the range of 140-160 mm Hg and/or diastolic (D) BP which fell in the range of 90-100 mm Hg. Exclusion criteria were secondary hypertension, diabetes mellitus, obesity or other associated diseases. We selected 95 normotensive males having a SBP and DBP of less than 140 and 90 mm Hg respectively, and matched them with the hypertensive males for age and body mass index (BMI). Both groups and the child population were Caucasians from St Petersburg.
In 109 EH patients (mean age 19,83±2,934; BMI 22,46±2,5) and 95 normotensive controls (mean age 22,01±2,323 ; BMI 22,15±1,85) and the child population we analyzed the frequency of the ApoE polymorphism (see Table) by means of endonuclease digestion. Allele frequencies and genotype distribution were compared by using χ^2 test.

Groups	Genotypes						Alleles		
	E2E2	E2E3	E2-E4	E3-E3	E3-E4	E4-E4	ε2	ε3	ε4
Controls n=95	1 1,05%	14 14,74%	2 2,26%	58 61,05%	18 18,95%	2 2,26%	0,095	0,779	0,126
EH males n=109	0 0%	12 11,05%	2 1,83%	78 71,56%	17 15,6%	0 0%	0,064	0,849	0,087
Children n=403	3 0,74%	67 16,63%	14 3,47%	243 60,3%	70 17,37%	6 1,49%	0,108	0,773	0,119

We have not found significant differences in allele and genotype distribution of the apoE polymorphism for either group.

P0945. Some Ethnic Features Of ACE And RIAT II Genotypes Distribution And Its Association With Arterial Pressure And Aorta Diameter

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Cases of atherosclerosis and essential hypertension are rare in natives of Far North. The reasons remain still unknown though this fact is statistically verified. Short stature of natives together with traditional nutrition, including great amounts of protein and fat and low amounts of carbohydrates associated with low blood insulin levels make these populations a special field of interest for researchers of the metabolic syndrome.
The object of this study was to analyze the ACE and RIAT II genes distribution and its association with arterial pressure values in geographically isolated group of Nenets children. Height, weight, arterial pressure (routine), aorta diameter (ultrasound) were measured and blood samples were obtained from 39 children - inhabitants of Polar Urals' tundra aged 6 - 12 years. To evaluate the I/D polymorphism of ACE gene we used the primers, flanking the polymorphous locus of 16 introne (Rigat B.C., et al.,1992). Data were compared with the data obtained from 403 European children living in Saint-Petersburg.
The frequency of I/I, I/D and D/D genotypes were found to be 0.51, 0.43, 0.05 in Nenets group compared to 0.25, 0.48, 0.27 in Europeans respectively, which is 5-fold lower for D/D. The frequency of RIATII C/C and A/C genotypes were found to be 0.85 and 0.15 in the group of Nenets children. Those Nenets children, who had I/I genotype had significantly higher systolic blood pressure (99.2 vs 92.9 mm Hg, $p<0.05$) and greater aorta diameter (12.7 vs 11.5 mm, $p=0.02$) compared to the others of the same age group.

P0946. Lack of support for linkage of a primary hip osteoarthritis locus on chromosome 6p

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Background: The most promising locus for primary hip osteoarthritis (OA) has been identified by Loughlin and co-workers within an

11.4cM region on chromosome 6p with a maximum multipoint LOD score (MLS) of 4.6 for microsatellite marker D6S1573. The region is adjacent to three candidate genes for OA; FBOX9, BMP5 and COL9A1. SNP analysis of BMP5 and COL9A1 has failed to show any evidence of linkage.
Objectives: To perform fine multipoint linkage analysis of this region using 109 Northern Irish primary hip OA pedigrees.
Methods: We examined 109 families, each containing at least one affected sibling pair concordant for hip OA, identified from operation lists for total hip replacement for primary hip OA. Microsatellite markers from the ABI LMS v 2.5 kit together with microsatellites for each candidate gene and other novel markers (produced in-house) were amplified using the multiplex PCR kit (Qiagen) and allelic genotyping performed using Genescan and Genotyper software (Applied Biosystems). Multipoint linkage analysis was performed using GENEHUNTER-PLUS.
Results: A total of 288 sibling pairs were included in the analysis; 180 females and 112 males. Table 1 shows the multipoint LOD scores (MLS) for the markers examined. None of these microsatellite markers demonstrated significant linkage.
Conclusion: We find no evidence to support the existence of a significant OA susceptibility locus on chromosome 6p.

Chromosome 6p multipoint analysis

Microsatellite	Chromosome map position (Mb)	Multipoint LOD score
D6S276	38.0	-2.11
D6S1549	46.6	-2.16
D6S282	48.2	-1.68
IL17	52.0	-1.50
IL17F	52.1	-1.48
FBOX9	52.8	-1.42
D6S1573	53.7	-1.34
D6S294	55.1	-1.16
BMP5	55.7	-1.12
D6S1276	55.8	-0.91
D6S257	56.0	-0.60
D6S223	56.2	-0.75
2326k(ca)	56.4	-0.92
2521k(gt)	56.6	-1.01
2767k(ca)	56.8	-1.25
D6S1557	70.8	-2.34
COL9A1	70.9	-2.87

P0947. Association of the CYP19 gene polymorphisms with risk of adenomyosis in North-West of Russia.

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Endometriosis and adenomyosis develop in women of reproductive age and regress after menopause or ovariectomy, suggesting that they grow in an estrogen-dependent fashion. Estrogen is suspected to be the most important known factor that stimulates the growth of endometriotic tissue. The conversion of C19 steroids to estrogens occurs in a number of tissues and this reaction is carried out by aromatase P450. The purpose of this study was to determine whether polymorphisms of two intronic restriction sites of estrogen receptor gene *ESR1 (Xba*I and *Pvu*II), a tetranucleotide (TTTA) tandem repeat polymorphism and a 3 bp I/D polymorphism in intron 4 of the aromatase gene (*CYP19*) contribute to endometriosis and adenomyosis as feasible genetic risk factors. 74 patients with endometriosis or adenomyosis and 90 healthy control women were used for PCR assay. The distribution of the genotypes and alleles of the TTTA repeat in intron 4 of CYP19 were not significantly different between patients and control groups. In contrast, an increased frequency of the D allele of CYP-19 was observed in the adenomyosis group compared to the control group (49% versus 26% p >0,001) and with the endometriosis group (49% versus 29% p >0,001). No significant association was found between these diseases and XbaI and PvuII polymorphisms of the ESR1gene, however XX genotype (XbaI polymorphism) and PP genotype (XbaI polymorphism) were more often encountered in the patient's groups. These results suggest that the 3 bp I/D polymorphism of the CYP19 gene may contribute to adenomiosis in a Russian population.

P0948. Genetic Form Of Reproductive Health

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The report outlines the concept of Genetic Form of Reproductive Health (GFRH) adopted for practical application in the Lab. for prenatal diagnostics of inherited and inborn disorders . GFRH summaries our 15 years experience in the management of women of child birth age as well as in predictive genetic testing for common multifactorial diseases which often complicate pregnancy and its outcome. According to GFRP the planning pregnancy couple is recommended for karyotyping and DNA testing for detection feasible carriers of major mutations in the genes responsible for the most common monogenic diseases (cystic fibrosis, phenylketonuria, spinal muscular atrophy, adreno-genital syndrome, Duchenne muscle dystrophy etc) . Genetic counseling after these tests should be supplemented with genetic testing of genetic polymorphisms associated with such common pregnancy complicating diseases as habitual miscarriages, placental insufficiency, endometriosis, preeclampsia, diabetes, some vessel diseases as well as nerve-tube defects in the fetus, susceptibility to some infections (cytomegalovirus, AIDs). Interpretation of relevant genetic information should be supplemented with personal obstetric recommendations and relevant presymtomatic treatment. The GFRH is most efficient if applied before pregnancy onset or at its earliest stages. It should be considered as useful supplement to already ongoing algorithm of prenatal diagnostics which usually starts after 10th week of pregnancy. Combined with adopted prenatal diagnostics service GFRH might be considered as useful supplement for improving management of pregnant women and the birth of healthy child.

P0949. The glutation-S-transferase T1 gene polymorphism in women with recurrent spontaneous miscarriages

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BACKGROUND: The pathogenesis of recurrent miscarriage is complex, presumably involving the interaction of several genetic and environmental factors. We investigated the relationship between recurrent spontaneous miscarriage and a polymorphism of the glutathione-S-transferase genes encoding responsible for xenobiotic conjugating enzymes of Phase II detoxification system (GSTT1). METHOD: In a prospective case-control study, 122 patients with a history of two or more unexplained first-trimester recurrent miscarriages (experimental group) and 76 women who had had one or more normal pregnancies and no obstetric complications or history of miscarriage (control group) were studied. We investigated the polymorphism of GSTT1 gene by PCR-RFLP analysis. RESULTS: Fifty seven of the 122 women of experimental group (46.7%) and sixteen of the 76 control women (21.1%, p<0.01) were homozygous for the glutathione-S-transferase T1 null allele (GSTT10/0).
The relative risk of the first-trimester recurrent miscarriages in carriers of the GSTT10/0 genotype was 3.29 (95% CI=1.73-6.24). CONCLUSIONS: These data support our previous finding on GSTT1 gene as a feasible genetic determinant of the risk of recurrent spontaneous miscarriage.

P0950. A new SNP in the CBFA1 promoter 1 region and its relation with lumbar spine BMD in Spanish postmenopausal women

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Osteoporosis is a multifactorial disease, with a strong genetic component. Several genes were reported to be involved in this pathology but there are controversial results and new candidates should be evaluated. Core binding factor α (Cbfa1) is a runt domain transcription factor that is essential for osteoblast differentiation, bone remodelling and fracture healing. Both knockout and overexpression of the CBFA1 gene in mice, result in an osteoporotic phenotype. Because of this, CBFA1 is a good candidate gene for association

studies in osteoporosis. A cohort consisting on 394 postmenopausal Spanish women (52.57 ± 7.5 years old) was available to evaluate this gene.
We have undertaken a search for new polymorphisms in the CBFA1 promoter 1, (constitutively used in immortalized human bone marrow stromal cells and osteoblast specific in rodents) and in the adjacent 5'UTR region. We have identified a new single-nucleotide polymorphism (allelic frequencies 83% and 13%) as well as an interaction between this polymorphism and age (p = 0.004). An association with lumbar spine BMD was only observed in a sub-cohort of 265 Spanish postmenopausal women over 50 years old (p = 0.007 after adjusting by body weight, body height and years since menopause). Gel retardation assays showed that oligonucleotides containing the polymorphic site specifically bound osteosarcoma (MG63) nuclear proteins.
In summary, this preliminary study describes a new SNP in the CBFA1 promoter 1 region, which may affect bone mass.

P0951. Genetic polymorphism of GSTP1 gene in Korean patients with preeclampsia

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Glutathione S-transferase P1-1 (GSTP1-1) is an important detoxification enzyme and the GSTP1-1 level has been found to be lower in placental and decidua tissue of preeclamptic women as compared with corresponding tissues of normal pregnant women. GSTP1 gene has a polymorphic site in exon 5 (Ile105Val) and this amino acid substitution causes a significantly lower enzyme activity and less effective capability of detoxification. We analyzed the allele frequency of Ile105Val in GSTP1 gene to determine the role of GSTP1 gene in the development of preeclampsia using PCR followed by restriction fragment length polymorphism (PCR-RFLP) method. We tested pregnant women with preeclampsia (n=85), their fetuses (n=90) and healthy controls (105 men and 101 women). The frequencies of genotype Ile/Ile and Ile/Val or Val/Val were 70% (n=60) and 30% (n=25) in pregnant women with preeclampsia, 71% (n=64) and 29% (n=26) in fetuses and 67% (n=137) and 33% (n=69) in healthy controls. There was no statistical significance between women with preeclampsia, fetuses, and healthy controls. In conclusion, the genetic polymorphism of exon 5 in GSTP1 gene may be less likely related to the development of preeclampsia in Koreans.

P0952. PARP-1 gene *Tai I* polymorphism and formation of the brain infarction in patients with carotid atherothrombotic ischemic stroke

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Poly(ADP-ribose) polymerasae -1 (PARP-1) is a nuclear enzyme that mediates early neuronal ischemic injury. We analyze the connection between *Tai I PARP-1* gene polymorphism, level of Poly(ADP-ribose)(PAR) in cerebrospinal fluid (CSF) and the brain infarction volume(IV) in 60 patients from Slavonic population with atherothrombotic ischemic stroke(IS). The presence of restriction site for *Tai I* was marked as(+), absence - as(-). PAR levels were assessed in CSF on days 1 and 3 after the stroke onset using ELISA technique. Slice-to-slice manual morphometry were used for calculation of the IV on days 1, 3, 7 and 21 after IS onset.
The comparison of the brain IV in patients with different *PARP-1* genotypes was performed using the γ-correlation analysis. A clear correlation between PARP-1 gene *Tai I* polymorphism and the infarction volume was observed. For instance, for day 1 γ = 0.31, p=0.02. In this point medium IV was significantly larger in patients with (-/-) genotype versus (-/+) and (+/+) carriers on day 7 after the stroke onset (p=0.0006). Furthermore statistical analysis revealed a strong and close association between CSF PAR levels and IV on day 3 after the stroke onset; in patients with high PAR levels large size infarctions were prevailed(p=0.05). Our results show the significant dependence of the IV on *PARP-1* gene *Tai I* polymorphism and the level of PAR in CSF. These data suggest that *PARP-1* is an important component of cell death pathway in brain ischemia.

P0953. Sequence variation in uroplakin 1b gene is associated with vesico-ureteric reflux

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Introduction. The uroplakin 3 (UPK3) knockout mice develop vesico-ureteric reflux (VUR) a finding that suggests the uroplakin genes (UPK1a, UPK1b, UPK2, UPK3) as possible candidate genes for VUR in humans. So far only the UPK3 gene has been screened for mutations but none were detected. Other uroplakin genes might be involved in the development of VUR as well. This study reports the results from screening of the coding regions of the UPK1b gene in patients with primary vesico-ureteric reflux.
Materials and Methods. The sample of 85 patients diagnosed with VUR was included in the study. Sequence variation screening of coding regions of the UPK1B gene was performed with the heteroduplex analysis followed by sequencing.
Results. A sequence variation changing asparagine to aspartic acid at the codon 137 (N137D) in exon 5 of the UPK1b gene was detected in 4 out of 85 (4.7%) patients with VUR and in 5 out of 600 controls (0.83%) (OR = 5.87, 95%CI 1.54-22.33, p= 0.017).
Conclusion. This result suggests that, in our sample of patients, the UPK1B gene appears to be associated with primary vesico-ureteric reflux. The UPK1B gene might be involved in the development of the disease in a small subgroup of patients with VUR.

P0954. Coronary artery disease and genetic polymorphisms in ABCA1, ACE, eNOS, ANG, APOAV, TP53, PON1 genes

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We have studied 85 patients with angiographically proven coronary artery disease (CAD) and 100 their brothers and sisters for polymorphisms of ACE, ANG, eNOS, p53, PON1, ABCA1, APOAV genes using S-TDT test and correlation analysis. No highly significant linkage of all genes under study with CAD, acute myocardial infarction, angina class were found accordingly to S-TDT test. In proband group R219K polymorphism of the ABCA1 gene correlated with angina severity, stenosis index. VLDL cholesterol level. In sibs group this polymorphism shows correlations with CAD, angina severity, VLDL cholesterol level . The data received on the association analysis between ABCA1 gene R219[[Unsupported Character - К]] polymorphism and CAD both in proband and sibs groups, indicate that allele K219 appears to be the risk factor of CAD development and the reason of complicated variant of CAD clinical course. Also, R1587K polymorphism ABCA1 gene shows correlation with CAD and myocardium infarction in sibs group. In proband group I/D polymorphism of the ACE gene correlated with the presence of silent ischemia. These patients tended to have higher rate of DD genotype. In sibs group this polymorphism shows significant correlations with CHD, acute myocardial infarction and angina severity . C[[Unsupported Character - А]]D patients from this group had significantly higher rate of DD genotypes of the ACE gene (40% vs 23% ones without C[[Unsupported Character - А]]D, F test [[Unsupported Character - р]]=0,0145, OR=2,25, CI 0,89-5,64). Other genes under study don't show significant level of correlation with clinical manifestations of coronary artery disease.

P0955. Possible Association of CD4 Gene Polymorphism with Vitiligo disorder in Iranian Population.

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Vitiligo is an acquired idiopathic and polygenic disorder characterized by the progressive circumscribed depigmented patches. The exact pathogenesis of the disorder is not yet known, but there are a series of studies which show that the ratio of CD4 positive T-cells alters in vitiligo patients and suggesting an autoimmune process

for development of the disorder. The CD4 gene plays an important role in the cell-mediated immune response and its association with the autoimmune IDDM has been previously reported, therefore we selected the CD4 gene as a candidate gene based on autoimmunity assumption of vitiligo pathogenesis. We studied the pyrimidine -rich pentanucleotide repeat polymorphism, located in the first intron of the CD4 gene. This region is very strategic and contains several regulatory elements for expression of CD4 gene. In the study, we screened 103 Iranian vitiligo patients (nonsegmental type) and 117 healthy matched controls, using PCR technique. Our results show that there is a significant association with allele A5 (p = 0.047) and increased and decreased frequency of the A4 (0.70 vs. 0. 56) and A7 (0.28 vs. 0. 39) alleles in cases compared with the controls respectively. We also found a border line protective role for A7/A8 genotype (P=0.0512). This study suggests that the CD4 gene polymorphism has a modest association with the development of vitiligo in Iranian patients and expanding the sample size would help to better clarify the association.

P0956. Association of different genetic polymorphisms with left ventricular hypertrophy in essential hypertension and arterial hypertension combined with diabetes mellitus type II

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Left ventricular hypertrophy (LVH) is a major independent risk factor for morbidity and mortality from cardiovascular disease. 11 genetic polymorphisms in seven genes (A-240T, A2350G in *ACE*; A1166C in *AGTR1*; C774T, G894T, VNTR polymorphism in intron 4 of *NOS3*; G-308A in *TNF*; C825T in *GNB3* and polymorphisms in genes of calcineurin pathway: -83 rpt in *PPP3CA* promoter; T-128C and A-131G in intron 4 of *GATA4*) were investigated in relation to cardiac hypertrophy in 135 Russian patients with essential hypertension (EH) and 94 with arterial hypertension, combined with diabetes mellitus type II (AH+DM2). Control sample consisted of 122 healthy individuals. Case/control study revealed association of RAS genes polymorphisms with presence of LVH in EH group and *NOS3* polymorphisms (C774T and G894T) in AH+DM2 patients.
Both polymorphisms of *ACE,* as well as *AGTR1* and VNTR of *NOS3* were associated with left ventricular mass index and wall thickness in patients with EH, whereas in AH+DM2 patients the association was found with C774T and G894T of *NOS3*. G-308A in promoter of *TNF* gene was connected with type of LV remodeling (LV remodeling index). Neither *GNB3,* nor calcineurin pathway genes polymorphisms were associated with cardiac parameters.
So, there are some differences in genetic predisposition to cardiac hypertrophy in arterial hypertension depending on the presence of DM2, which is should be take into account in future genetic studies and therapy.

P0957. Systematic linkage disequilibrium analysis of SLC12A8 at PSORS5 in Psoriasis and Psoriatic Arthritis

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The gene for soluble co-transporter SLC12A8 has recently been proposed as a candidate gene for psoriasis susceptibility (PSORS5) on chromosome 3q based on association of various SNPs in a Swedish cohort. We attempted to replicate this finding in 210 German trios with psoriasis but failed to identify significant association. Since in a previous study linkage to this locus was stronger when families were stratified according to “joint complaints”, we hypothesized SLC12A8 might be a susceptibility locus for psoriasis arthritis rather than psoriasis vulgaris. We therefore recruited new cohorts of single patients: 341 with psoriatic arthritis and 282 with psoriasis vulgaris without joint affection. We determined the haplotype structure of SLC12A8 through systematic linkage disequilibrium analyses of 49 SNPs and grouped them into seven LD-blocks of 2-14 kb size each. Next we investigated for association in a case-control study using 27 htSNPs. We detected association of 1 single SNP and 2 LD-blocks in the psoriatic arthritis cohort (chi-square 10.7, p=0.001 and 5.96, p=0.015, respectively). In the case cohort of psoriasis vulgaris 3 single SNPs (chi-square 14.6, p=0.00013) and the same 2 LD-blocks also showed association (chi-square=14.2, p=0.00017).
Based on these data, SLC12A8 seems to be a susceptibility locus not only for psoriatic arthritis, but also for psoriasis vulgaris. The hypothesis that psoriatic arthritis might be an independent disease entity could not be confirmed and remains to be resolved. In order to identify the exact nature of this association, identification of the disease-causing variant and functional studies are required.

P0958. Identification of Candidate Genes in Patients with Amyotrophic Lateral Sclerosis by Breakpoint Characterization

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons in the brain and spinal cord. Familial forms of ALS (FALS) account for 5-10% of all ALS patients and to date two disease genes, SOD1 and ALS2, have been identified.
Genetic causes of the sporadic forms of ALS (SALS) are still unknown.
We have investigated five patients with SALS carrying balanced chromosomal rearrangements (t(4;19)(q22;p13.1), t(4;20)(p15.3;q11.2), t(18;21)(q23;q22.1), inv(12)(p11q13), inv(X)(p11.2q21.3)) in order to identify SALS underlying genetic factors.
By fluorescence in situ hybridization, we have narrowed all breakpoints to intervals of 100 kb or less. In three patients the breakpoint regions harbour interesting candidate genes that may have an impact on the aetiology of SALS.
In the case of the inversion 12, multiple candidate genes have been found, including ITGA7, which encodes the integrin alpha chain 7, GCN5L1 encoding the general control of amino-acid synthesis 5-like 1, and the uncharacterized ARG99 gene. In the translocation t(18;21), the chromosome 21 breakpoint interval of 70 kb contains the GRIK1 gene, a glutamate receptor subunit whereas molecular analysis for 18q23 did not provide an indication for a disrupted gene. In the translocation t(4;19) fine-mapping of the breakpoints by Southern hybridization showed that on each site, an as yet uncharacterised transcript may be affected by the rearrangement. Further characterization of these transcripts and mutation analysis in identified candidate genes will soon shed more light on their relevance in SALS.

P0959. Vitamin D receptor gene 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 well-known phenomenon in children with juvenile chronic arthritis (JCA). The bone abnormalities in these patients include juxta-articular OP (early radiographic changes), systemic OP and growth retardation. Previous studies have suggested an influence of vitamin D receptor (VDR) alleles on bone metabolism (peak bone mass) in patients with JCA.
The aim of our study was to investigate the relationship between VDR gene polymorphism and BMD in children with JCA.
Fifty three JCA children (36 girls, 17 boys) were included in our study. The mean age of patients was 10.6 ±2.6 years. The TaqI and ApaI polymorphisms of the VDR gene and serum levels of intact osteocalcin, calcitonin, Ca^{2+}, phosphate, and parathyroid hormone were tested in all patients. OP was detected by dual-energy X-ray absorbtiometry in the lumbar spine (L1-L4).
Using the data of BMD we divided the children into two groups: with

OP - 30 children (56.7%) and without OP - 23 children (23.3%). There were significant differences between polymorphism of the VDR gene and BMD in those groups. Also we investigate the tendency to sex differences between polymorphism of the VDR gene and BMD JCA boys and girls with OP and without OP.

Groups	Genotypes					
	TT	Tt	tt	AA	Aa	aa
JCA children with OP (n-30)	15 50%	15 50%	0 0%	1 3.3%	17 56.7%	12 40%
JCA children without OP (n-23)	8 34.8%	12 51.2%	3 13%	6 26.1%	11 47.8%	6 26.1%
p	<0,01			<0,01		

P0960. Subarachnoid Hemorrhage: Roles of *ApoE* and *ELN* genes

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Subarachnoid Hemorrhage (SAH) secondary to aneurismal rupture is a complex disease, with an annual incidence of 6-10 per 100,000 and a 30 day case mortality rate of approximately 40%. There is strong evidence that both genetic and environmental risk factors are involved in its etiology. Apolipoprotein E (*ApoE*) and Elastin (*ELN*) genes may be important candidates in pathogenesis of SAH. Genetic variants in *ApoE* have been associated with lipid profile and cardiovascular disease. Similarly, polymorphisms in *ELN* have been implicated in cerebral aneurysms. However, these associations remain inconclusive. We performed a case-control (age, race, gender matched) association study of SAH using 10 single nucleotide polymorphisms (SNPs) in the *ApoE* gene and 8 SNPs in the *ELN* gene in a sample of 138 cases and 267 controls of Caucasian and African-American descent. Haplotype analysis revealed significant association with *ApoE* (Caucasians p = 0.0001, African-Americans p = 0.001). However, in *ELN*, no association with SAH was found at allelic or haplotype level in either ethnic group. Also, there was no association of *ELN* variants with intracranial aneurysm (IA) cases; however, there was even stronger association of *ApoE* polymorphisms with IA subset of the cases. This is the first study to examine these regions for cerebral aneurysms among Caucasians and African-Americans in the United States. While our results do not confirm prior reported associations with *ELN*, our study indicates that *ApoE* may play an important role in the pathogenesis of SAH.
Supported by US National Institutes of Health grants NS36695 and ES06096.

P0961. Genotype distributions of ACE and angiotensin type 2 receptor genes in Italian children with congenital uropathies.

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ACE and AT2R gene polymorphisms have been associated with an increased incidence of congenital anomalies of the kidney and urinary tract (CAKUT). We investigated the genotype distribution of these polymorphisms in Italian children with CAKUT. Besides, we evaluated the association between the ACE I/D and the AT2R gene polymorphisms with the progression of renal damage in subgroups of CAKUT patients. We recruited 102 CAKUT Italian children, 27 with vesicoureteral reflux (VUR), 12 with hypoplastic kidneys (HK), 20 with multicystic dysplastic kidneys (MCDK), 13 with ureteropelvic junctions (UPJ) stenosis/atresia, 18 with nonobstructed, nonrefluxing primary megaureters (MU) and 12 with posterior urethral valves (PUV) compared with 92 healthy controls. ACE and AT2R gene polymorphisms were analysed by PCR. The identification of AT2R gene polymorphisms in intron 1 and in exon 3 was revealed by enzymatic digestion. ACE genotype distribution in CAKUT was no different from the one of the controls, but the subgroup of patients with radiographically renal parenchymal abnormalities showed an increased occurrence of the D/D genotype. The frequency of the G allele of AT2R gene in CAKUT was increased in respect to that of the controls. By contrast no significant difference in the frequency of the C and A alleles of the AT2R gene was found.Our findings indicate that the ACE gene can be a risk factor in the progression of renal parenchymal damage in CAKUT patients. Moreover, a major role of the AT2R gene in the development of CAKUT has been found, at least in Italian children.

P0962. Linkage and haplotype analysis for chemokines receptors cluster in chromosome 3q21.3 transmitted in family pedigree with asthma and atopy

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Background: Genomic scan analyses suggested that chemokines cluster (CCR2, CCR3, CCR5 < 300 kb span) in chromosome 3 may contribute to HIV-1 infection and other inflammatory diseases. Two SNPs and deletion in these chemokines respectively may contribute to asthma and related phenotype.
Objectives: Identifying if these polymorphisms are in linkage and linkage disequilibrium to asthma. Comparing the benefit and the relative advantage among family-based association tests (FBATs), linkage analyses, and haplotype analyses.
Material and methods: Nuclear families (n=152, 453 unrelated individuals) including 303 unrelated parents, and 150 unrelated children. Asthma was defined as physician diagnosed asthma (PDA). Atopy defined as skin prick test (SPT ≥ 3 mm) to common inhaled allergen. These polymorphisms -64V/I, -17T/C and a 32 bp deletion respectively were genotyped using TaqMan assay and PCR method.
Results: We observed highly significant output with PDT but not with TDT for FBATs. Only 13 families were eligible for linkage analysis, but 152 families for haplotype analysis. Both analyses confirmed that these chemokines are in linkage to asthma, but not atopy. Protein expression analysis confirmed that these polymorphism affect on the expression of these receptors.
Conclusion: PDT is more powerful than TDT as it include more informative data and less affected by genetic pentrence. Haplotype analysis included more informative data but with no significant different of the outcome with the linkage analysis. The affect of the polymorphism on the protein expression suggested new plausible pathway of these chemokins on the inflammatory diseases.

P0963. Trisomy 7 in the synovial fluid cells of patients with rheumatoid arthritis

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Objective: Recent studies revealed trisomy 7 as a chromosomal abnormality in non-neoplastic disorders such as rheumatoid arthritis (RA). In the present study, we investigated the presence of trisomy 7 in the synovial fluid cells of patients with RA using fluorescence in situ hybridisation (FISH) analysis.
Methods: Synovial fluid from 15 patients with RA was collected of knee joints. The control group consisted of seven patients with traumatic synovial effusion in their knee joints. The arthrocenteses were performed under aseptic conditions. Dual colour FISH analysis by using chromosome 7 specific LSI D7S522 (7q31) and chromosome 5 specific LSI EGR1 (5q31)/D5S721 (5p15.2) probes on the slides prepared from synovial fluid of RA patients and controls.
Results: The slides of our cases were analysed by using two different DNA probes. When the slides hybridised with chromosome 5 specific probes were analysed, no trisomic or monosomic cells were revealed in both patients and controls. However in 8 of 15 patients, trisomy 7 occurred in a variable percentage of cells (23% up to 48%) of synovial fluid. No monosomy 7 cells were detected in these specimens. All control cases were disomic for chromosome 7.
Conclusion: The results of the present investigation suggest that trisomy 7 may play a role in the pathogenesis of synovial hyperproliferation in RA.

P0964. Genome scan for type 2 diabetes in genetically isolated Dutch population reveals interaction between chromosomes 5 and 18

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Multiple genes, interacting with the environment, contribute to the susceptibility for type 2 diabetes mellitus (DM2). We performed a genome-wide search to localize DM2 susceptibility genes in a recently genetically isolated population in the Netherlands. In our data, the regions 3p, 3q, 5, 18, 19 and 5 previously identified by other groups demonstrated a LOD>1.18 (nominal P<0.01). Also, we identified a pedigree in which two segments, one at chromosome 5 and other on chromosome 18, co-segregated in a perfect manner with each other and the disease status. This finding was highly significants: the probability to have these haplotypes co-segregating by chance was 2.8 10^{-7}. Except confirmation of previous findings and an example of interaction in DM2, these findings prove empirically the utility of our population for mapping genes for complex disorders.

P0965. An intronic G>A substitution but not the Lys167Asn mutation in Lox-1 gene is associated with the risk of coronary artery disease

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BACKGROUND: Coronary artery disease (CAD), in contrast to rare monogenetic diseases, has been regarded as a complex disease affected by environmental and genetic factors. Lectin-like oxidized low-density lipoprotein receptor (LOX-1) has been suggested to promote the occurrence of CAD. Recent works reported the presence of several SNPs both in intronic, exonic and 3'UTR region in LOX-1 gene. The aim of our study is to examine the association of two SNPs with risk of CAD.
METHODS: DNA of 72 early CAD patients (age<55) and 72 controls age and sex-matched was extracted to examine the G501C polymorphism in exon 4 that results in a missense mutation (Lys167Asn) and a G>A substitution in intron 4 located 14 bp from the 5' end of exon 5.
RESULTS: When we compared the group of CAD with the controls we found that the patients had a significantly different distribution of intron 4 G>A variant compared with the controls (chi²=10.23; DF=2; p=0.0069). There was no significant association between K176N distribution among two groups.
DISCUSSION: Thus, we conclude that the common intronic variation of the LOX1 gene may be associated with the risk of CAD. No assocation was found for K176N polymorphism, recently reported by Tatsuguchi M. et al. as an important marker for myocardial infarction in Japanese population. The exact mechanism by which LOX-1 can affect the risk of CAD is not clear. Probably throught direct effect on the metabolism of oxLDL.

P0966. Association between the microsatellite polymorphism $(TTTTA)_n$ in the promoter of the *CYP11A* gene and ovarian hyperstimulation syndrome

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The aim of the study was to investigate an association of the $(TTTTA)_n$ microsatellite polymorphism in the promoter of the *CYP11A* gene with ovarian hyperstimulation syndrome (OHSS) and with the degree of androgenemia during controlled ovarian hyperstimulation (COH).
Fifty-eight non-polycystic ovary syndrome (non-PCOS) patients at high risk of OHSS and 58 control patients without signs of risks of OHSS during COH were enrolled in the study. The *CYP11A* $(TTTTA)_n$ allelic variants were genotyped and total serum testosterone (T) levels determined.
We confirmed the existence of four different types of *CYP11A* alleles, alleles with four, six eight or nine TTTTA repeats. The allele frequencies were 0.51, 0.26, 0.14, 0.09 in the study group and 0.49, 0.29, 0.11, 0.10 in the control group, for alleles with four, six, eight and nine repeats, respectively. The difference in the allele distribution between both groups was not statistically significant (P = 0.888). The distribution of the 4+ genotype (with at least one copy of the four-repeat-unit allele) and 4- genotype (without the four-repeat-unit allele) was identical in the two groups. Normoandrogenemia was found in all patients. There was no statistically significant difference in the distribution of total serum T levels within the study (P = 0.111) and control (P = 0.327) group of patients having different genotypes. Our results suggest that in Slovene non-PCOS patients undergoing COH, an association of the $(TTTTA)_n$ microsatellite polymorphism in the promoter of the *CYP11A* gene with OHSS and with total serum T levels during COH could not be confirmed.

P0967. Two new highly polymorphic markers in the 3' UTR region of the PLA2G7 Gene

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Lipoprotein-associated phospholipase A_2 (Lp - PLA_2) or platelet activating factor acetylhydrolase (PAF-AH), is involved in the degradation of PAF and oxidazed phospholipids and may play a role in inflammatory diseases. High levels of Lp - PLA_2 have been demonstrated to be an indipendent risk factor in coronary artery disease (CAD). In this report we describe two new highly polymorphic markers located 31bp downstream of the last nucleotide of exon 12 in the 3' UTR region of the gene $1344+31TG_n\,AG_m$. The polymorphic regions consist of two consecutive dinucleotide repeats: $TG_{n(10\text{-}17)}$ and $AG_{m(10\text{-}23)}$. Eigth alleles were observed for the TG repeat, the most common alleles carrying 12 and 15 repeats. Fourteen alleles were identified for the AG dinucleotide repeat, the most frequent alleles carrying 12-13 repeats. Heterosigosity for this microsatellites was 82.5% and 85% for the TG_n and the AG_m repeats, respectively, in North Italian individuals. These markers may be very useful in linkage or association studies of the PLA2G7 gene in inflammatory diseases or other diseases were Lp - PLA_2 may play a role in the pathogenesis. No association was found in a small case-control study with coronary artery disease patients and controls.

P0968. Alterations in Brain-derived Neurotrophic Factor (BDNF) plasma levels in patients with Eating Disorders

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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 Met66 allele with anorexia and bulimia and the experiments performed in murine models of disruption of the Bdnf gene support the view that increased levels of BDNF could be involved in restricting food intake and low body weight. We hypothesize that ED are characterized by high BDNF levels in several regions of the central nervous system, leading to consequences in eating behavior. We assessed the BDNF plasma levels using an ELISA method and compared them between ED patients (n = 133) and control subjects (n = 68). We have found that BDNF levels were significantly higher in ED patients than in controls (43.5 ng/ml vs. 16.9 ng/ml; P = 0.003). No significant differences were found between subgroups of ED patients (restricting anorexia nervosa (ANR), binge-eating anorexia nervosa and bulimia nervosa (BN)). We also compared plasma levels between ED subjects carrying the -270C and Met66 alleles of the -270C/T and Val66Met BDNF SNPs. While not significant effect of the Val66Met polymorphism was observed, the BDNF plasma levels of the -270C carriers were significantly higher that those of non-carriers (45.3 ng/ml vs. 27.0; P = 0.003). These results support our previous association findings and further indicate that BDNF is involved in the susceptibility to ED.

P0969. Vitiligo Susceptibility And T/C Single Nucleotide Polymorphism In Exon 9 of the Catalase Gene

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Vitiligo is an acquired idiopatic hypomelanotic disorder due to melanoncyte death with a prevalence of 1-2 % world wide. Although the cause of melanoncyte death remains unclear, the oxidative stress appears to be an important phenomenon in the pathophysiology of vitiligo. The accumulation of hydrogen peroxide (H_2O_2) and low catalase levels have recently been demonstrated in the epidermis of vitiligo patients.

The aim of this study is to find out possible association between the T/C single nucleotide polymorphism (SNP) in exon 9 of the *CAT* gene and vitiligo susceptibility in Turkish population.

In 34 vitiligo patients and 49 healthy controls T/C single nucleotide polymorphism (SNP) in exon 9 of the *CAT* gene was analysed using the PCR-RFLP method (restriction endonuclease *Bst*X I).

It was found that there was no statistical difference between the frequency of T/C single nucleotide polymorphism in vitiligo patients and normal controls. The observed genotype frequencies of control and vitiligo patient groups differ significantly from that predicted by the Hardy-Weinberg equation for Caucasian population. The polymorphism, which is located in the CAT gene and thought that possible association with vitiligo susceptibility in other populations, can not be have an important role in etiology of vitiligo in Turkish population. To confirm this we need further support researches with wide groups.

P0970. A study of vitiligo sensitivity using DNA repeat polymorphisms on T-lymphocyte antigen-4 (CTLA-4) gene

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The Cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene encodes a T-cell receptor that is involved in the regulation of T-cell activation. Recent studies have demonstrated an association of a microsatellite polymorphism [variant lengths of a dinucleotide $(AT)_n$ repeat] lying in exon 3 of the CTLA-4 gene, with autoimmune disorders, such as Graves' disease, autoimmune Addison's disease and autoimmune hypothyroidism. The aim of the present study was to determine whether the same polymorphism of the CTLA-4 gene was associated with vitiligo. CTLA-4 gene microsatellite polymorphisms were determined for 36 vitiligo patients (21 female, 15 male) and 100 healthy controls by polymerase chain reaction amplification of genomic DNA and resolution of the products on polyacrylamide sequencing gels. The frequency of the A9 (96-bp) allele was significantly increased (P = 0.019) in vitiligo patients as a whole, in comparison with control subjects. This result suggests that there could be an association between the A9(96-bp) allele and vitiligo.

P0971. Mutation screening studies on IMMP2L and LRRN3 in Tourette syndrom and autism

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We previously reported the disruption of the inner mitochondrial membrane peptidase 2 -like (IMMP2L) gene by a breakpoint in a male patient with Tourette syndrome (TS). In the present study we sought to identify genetic variation in IMMP2L which might contribute to the manifestation of TS. We therefore screened for IMMP2L mutations in leukocyte DNA of 30 TS patients and unaffected controls. Due to its localisation in the critical region for autistic disorders (AD) on chromosome 7 mutation screening of IMMP2L was also performed on about 100 multiplex autism families. We analysed the entire coding sequence of IMMP2L, including intron-exon boundaries, by DHPLC analysis and subsequent direct sequencing of PCR-amplified fragments. No mutation except an intronic polymorphism in one of the autism patients was found. Somatic cell hybrids containing human chromosome 7 and human cell lines carrying a maternal uniparental disomy for chromosome 7 (mUPD7) were used to determine whether IMMP2L on chromosome 7p31 is imprinted in humans. Additional sequence comparison analysis for the coding region of IMMP2L confirmed the highly conserved character of this gene. In addition mutation screening was also performed on the brain expressed LRRN3 gene located in intron 3 of IMMP2L.

P0972. The analysis of the IL4 and IL9 polymorphisms in asthma patients and healthy donors from Volga-Ural region of Russia.

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Asthma is a common chronic respiratory disease, the development of which is determined by the interaction between genetic and environmental factors. Chromosome 5q31-34 contains multiple candidate genes for asthma including the cytokine gene cluster. Cytokines play an integral role in the coordination and persistence of the allergic inflammation of the airways in asthma. This study reports the results of the IL4 and IL9 polymorphism analysis in asthma patients and healthy donors from Volga-Ural region of Russia. The asthma group consisted of 140 patients with different ethnic origins, the control group included Russians (57 individuals), Tatars (57) and Bashkirs (50). The genotyping of SNP polymorphisms was performed by PCR followed restriction analysis. We have detected significantly differences of allele and genotype frequencies of -590C/T polymorphism of the IL4 gene between Tatar and Bashkir populations, Tatars and Russians ($p<0,05$). The frequency of TT genotype was higher in Bashkirs (14%) compared with Russians (0,7%) and Tatars (1,8%). In asthma patients the genotype frequencies were 54% for CC, 41% for CT and 5% for TT. We have not determined essential distinctions between asthma patients and healthy individuals. The analysis of the IL9 gene was not confirmed the association of the T113M polymorphism with asthma. An allele and genotype frequencies distribution was similar in asthma patients and control group. The data of this study revealed differences in the distribution of polymorphic alleles and genotypes of the IL4 gene between different ethnic groups, but not detected significantly differences between asthma patients and healthy donors.

P0973. Hereditary Congenital Hearing Loss: Mutation Analysis of Connexins 26 And 30 In Italian Population.

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Mutations in GJB2 gene are responsible for a majority of non-syndromic recessive deafness (NSRD). More than 100 different mutations are described but one is particularly common, the 35delG (about 60% of mutated *GJB2* alleles). We analysed 350 NSRD patients and identified mutations in 228/700 chromosomes; 21.8% (153/700) showed 35delG, while the remainder showed 23 different mutations. Five out of these mutations were novel: H100L, A40G, W133X and C174R were recessive while the D179N mutation

showed a dominant segregation. We also found three allele variants: V27I, V153I and G160S and a novel one 684 C→ A in the 3'UTR of unknown significance. 35delG was present in about 71% of all Cx26 mutations identified. Since other connexin genes are involved in non-syndromic deafness, we investigated the *GJB6* gene, encoding Cx30, for the large deletion including exon 1 of *GJB6*, del(GJB6-D13S1830), that is the cause of deafness in patients carrying one recessive mutation in the *GJB2* gene in *trans*. 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 account for less than 30% of NSRD cases and confirm that the 35delG mutation is the most frequent one, but many other are present such as M34T, E47X, L90P and delE120. Moreover 5 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.

P0974. Linkage analysis reveals novel locus for atopic dermatitis on chromosome 19p

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder and a major manifestation of allergic disease. In the industrialised countries the prevalence of AD is approximately 15%, with a steady increase the last decades. Two genome-wide linkage studies revealed a striking overlap of linkage regions for AD with known loci for a distinct chronic inflammatory skin, psoriasis, on 3q21, 1q21, 17q25, and 20p. We therefore investigated the psoriasis locus PSORS6 on 19p as a candidate region for AD.
i) Genome-wide linkage analysis for AD, performed in 199 European affected sib pair (ASP) families, revealed modest evidence for linkage on 19p (Z_{all} 1.94, p=0.026) with a significant increase (Z_{lr} 2.70, p=0.0035) when weighting the families 0 or 1 according to their linkage contribution to a major AD locus on 3q21 that was identified in the same cohort. ii) Fine mapping on 19p in the original family cohort using additional microsatellite markers confirmed the linkage. iii) To further establish the results, a secondary set of 84 European ASP families with infantile AD was analysed. This data set revealed suggestive evidence for linkage on 19p (Z_{lr} 2.67, p=0.0035) under the same weighting model for the AD locus on 3q21 thus providing independent confirmation of both linkage on 19p and the interaction with 3q21. While AD and psoriasis are distinct clinical entities that show no epidemiological association, the newly identified candidate region may contain genetic variants specific to skin barrier function and immunity and may thus facilitate the identification of the underlying disease genes.

P0975. CYP2D6 Deficiency Causing Severe Side Effects in Antidepressive Therapy (Case Report)

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Response to medication is characterized by a wide individual heterogeneity of side effects and efficacy of treatment. Genetic polymorphism is an important factor contributing to this phenomenon. Many antidepressants are metabolized at least in part by Cytochrome II D6 (CYP2D6) which exhibits marked differences in its catalytic activity. Several allelic variants of the CYP2D6 gene are associated with enzyme deficiency or total absence of the enzyme activity, whereas amplification of the CYP2D6 gene results in ultra fast metabolism of substrates. Depending on the number of functional alleles, the phenotypes can be classified in poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultra extensive metabolizers (UM). Approximately 7% of Caucasians exhibit a PM phenotype, thus being susceptible to develop adverse drug reactions when treated with standard doses of various antidepressants.
A 51 year-old female was treated for depression with Mirtazapine, Venlafaxine, Trimipramine and Reboxetine in standard doses. After one week she developed tachycardia, ventricular extra systoles and restlessness. Liver and kidney function was normal. DNA analysis of the CYP2D6 gene demonstrated homozygosity for the CYP2D6*4/*4 genotype which is characterized by a G1846A splice-site polymorphism. The CYP2D6*4 allele results in a complete loss of enzyme activity.
Since Mirtazapine, Venlafaxine, and Trimipramine are substrates of CYP2D6, the dose was adjusted according to the genotype. Depression symptoms were suppressed and side effects disappeared. In conclusion genotyping of CYP2D6 is a useful tool for detection of PM individuals and suitable for dose adjustments.

P0976. Analysis of the CYP19 gene polymorphisms in endometriosis patients from the North- West of Russia.

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Endometriosis is a complex disorder that is characterized by the presence of endometrial tissue in ectopic sites outside the uterus. Endometriotic stromal cells aberrantly express aromatase (CYP19 gene), which converts C19, steroids to estrogens. To investigate whether polymorphisms of CYP19 genes are associated with the risk of endometriosis, we analysed the frequency and distribution of a tetranucleotide (TTTA) tandem repeat polymorphism and a 3 bp I/D polymorphism in intron 4 of the CYP19 gene. 117 patients with clinically, endoscopically and histologically proved endometriosis and 90 healthy control women were included in the study. All patients were subjected to complex operative and conservative treatment. 43 patients (group 1) revealed positive responses to combined surgical and therapeutic treatment. The remaining 62 women demonstrated conspicuous resistance to this kind of treatment and were arbitrarily attributed to the group 2. The genotypes and alleles distribution for TTTA repeat-polymorphism as well as I/ D polymorphism were not significantly different between patients and control groups. In contrast, an increased frequency of the D allele was observed in the endometriosis group 2 compared to this one in the group1 (32% versus 17% p >0,01). Carriers of the D allele were registered in 55% of the endometriosis group2 compared to 28% of the group1 (p >0,01). These findings suggest that 3 bp I/D polymorphism of the CYP19 gene as well as earlier discovered null -genotypes of the both GSTM1 and GSTT1 genes might be associated with treatment efficiency of endometriosis patients in a Russian population.

P0977. The Thr92Ala SNP in the DIO2 gene is associated with a reduced risk of type 2 diabetes

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Type 2 diabetes (T2D) exerts a tremendous burden to health care systems. It recognizes a genetic background which, to date, is mostly unknown. Since insulin resistance and/or obesity play a major role in the pathogenesis of T2D, genes involved in the regulation of insulin sensitivity and/or metabolic rate and body weight, are likely to modulate T2D risk. Deiodinase 2 (DIO2) is a selenoenzyme that, by converting T4 to T3 via 5' deiodination, may play a role in modulating the metabolic rate and body weight. Recently a missense SNP (Thr92Ala) of the DIO2 gene has been associated with insulin resistance and obesity in women from a Caucasian population. We genotyped the Thr92Ala SNIP in a Caucasian cohort of 495 T2D patients and 598 controls from the Gargano region (East Coast of Italy). The X92A genotype (both T92A and A92A) was less frequent (p=0.02) among patients with T2D (263/495, 53.1%) than among controls (381/589, 63.7%): O.R. (and 95% C.I.) being 0.6 (0.4-0.9) after adjusting for age and gender. No major changes O.R.= 0.6 (p=0.04) were observed after adjusting also for BMI. In conclusion, our data demonstrate that among Caucasians from the Gargano region the X92A genotype of the DIO2 gene is associated to reduced

risk of T2D. Further functional studies will clarify whether the Thr92Ala SNIP has biological relevance which makes it an etiological genetic variation or it is a marker in linkage disequilibrium with other causative mutation(s) located in the same DIO2 gene or in other flanking genes.

P0978. Evaluation of SPINK5 gene polymorphisms as susceptibility factors for common allergic disease

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Mutations in the *SPINK5* gene, on chromosome 5q32 and coding for LEKTI, a serine protease inhibitor, have been identified to be disease causing in Netherton syndrome. Atopic manifestations including high levels of serum-IgE, eczema, asthma and hay-fever form part of this severe syndrome. Coding polymorphisms in the *SPINK5* gene have also been implicated in the development of common allergic disease. A recent study showed that maternally inherited alleles for the two polymorphisms Asn368Ser and Glu420Lys in *SPINK5* are associated with the development of atopy, atopic dermatitis (AD), and total serum-IgE concentrations. The association with the maternal allele Lys420 was confirmed in an independent cohort.
The goal of this study was to evaluate the *SPINK5* polymorphisms in two family cohorts of European origin that were independently recruited for two of the phenotypes, AD and atopy, previously associated with *SPINK5*. The GENUFAD-cohort consists of 199 affected sib-pair (ASP) families with severe infantile AD, while the MISS-cohort includes 99 ASP families with sensitisation against house-dust mite. Six microsatellite markers surrounding *SPINK5* and four coding polymorphisms (Asn368Ser Asp386Asn, Glu420Lys and Glu825Asp) were genotyped and analysed for linkage and association with AD and allergic sensitisation. No linkage was detected in the *SPINK5* region by non-parametric or parent-specific linkage analysis (Genehunter-Imprinting). Neither was any association of the alleles Asn368 or Lys420 with any of the clinical phenotypes observed. We conclude that SPINK5 polymorphisms may not be a general susceptibility factor for common allergic disease.

Cohort	Marker	Phenotype	Transmissions (T:NT)	p-value	Maternal (T:NT)	p-value
Genufad	Glu420Lys	AD	217:212	0.81	78:76	0.82
Genufad	Glu420Lys	IgE*	145:148	0.86	51:52	0.86
MISS	Glu420Lys	Allergic sensitization**	105:80	0.25	39:35	0.59
Genufad	Asn368Ser	AD	205:215	0.63	71:77	0.67
Genufad	Asn368Ser	IgE*	142:145	0.86	47:51	0.68
MISS	Asn368Ser	Allergic sensitization**	83:102	0.11	34:37	0.71

* either elevated total-IgE or specific sensitization
** sensitization against house-dust mite

P0979. A potential role of Toll-like receptor 3 (TLR3) for asthma

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Virus infections in early life is a risk factor to develop asthma symptoms in adulthood. TLR genes are Toll-like receptors which function is the immune pattern recognition for bacterial motifs. Toll-like receptor 3 (TLR3) recognizes double-stranded RNA (dsRNA), pattern associated with viral infection, because it is produced by most viruses during their replication. TLR3 is expressed in the airway epithelium and TLR3 activation induces the activation of NF-kB and the production of type I interferons and chemokines (i.e. RANTES). By means of Pyrosequencing technology we have studied two SNPs in the TLR3 gene with a minor allele frequency major than 10% (L412P (rs3775291) and P459P (rs3775290)). We have analyzed a sample of 50 asthmatic children patients and 100 unrelated individuals from the general population. Allelic frequencies were in H-W equilibrium and no differences in the allelic distribution were found. No allelic frequencies were available for the P459P in the public databases, but now we can confirm these polymorphisms in a sample of 180 unrelated chromosomes from the Spanish general population (MAF=0.3). The L412P variant showed a significant association to asthma in our sample in a recessive trait comparison (Phe/Phe) (F=5.45, two-sided p=0.03). Both changes are located in the extracellular leucine-rich repeats domain and alterations in this domain could act by stimulating or blocking the receptor function in a unknown manner affecting the host defence barrier. In this way TLR3 agonist (as CpG ODN) has been shown to drive towards induction of Th1-type response.

P0980. Identification of a novel candidate region for sporadic amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterised by progressive selective destruction of motor neurons. The disease is familial in 10% of cases, exhibiting an autosomal dominant inheritance. Mutations in the Cu/Zn superoxidase gene (SOD1) account for approximately 20% of individuals with FALS. Little is known regarding the pathogenesis of the sporadic form of ALS (SALS), which occurs in the remaining 90% of individuals.
Studies of the APE gene (APEX) on chromosome 14 in the Scottish and Irish ALS populations identified the D148E polymorphism, which shows an allelic association with sporadic ALS. The APEX locus lies in close proximity to the angiogenin gene, chromosome 14q11.2. This candidate gene shares a number of functional features with VEGF, which is an important angiogenic factor with neuroprotective properties. VEGF has been demonstrated to be a modifier of ALS in both mice and humans (Nat. Genet. **34** 383-394 2003). We identified an at-risk SNP in the angiogenin gene for the Irish sporadic ALS population.
The discovery of a SNP in the angiogenin gene which confers a significantly greater risk of the development of sporadic ALS provides new evidence towards the angiogenesis model suggested by studies of the VEGF gene. The observation of two significant allelic associations in genes of close physical proximity within the same population suggests the q11.2 region on chromosome 14 as a new candidate region for the sporadic form of the disease.

P0981. Exon 1 and promoter single nucleotide polymorphisms of the CTLA-4 gene in Myasthenia Gravis patients from Bashkortostan, Russia

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The autoimmune process in Myasthenia Gravis (MG) results in postsynaptic blockade of neuromuscular junction by autoantibodies directed against the acetylcholine receptor (AChR). The production of autoanti-AChR antibodies in MG is dependent on T-cell help. A polymorphism in the gene encoding cytotoxic T lymphocyte-associated antigen 4 (CTLA4) has been reported to alter T cell activation and confirm genetic susceptibility to several autoimmune diseases including MG. To determine the role of CTLA4 polymorphism in predisposition to MG we investigated 101 patients with MG and 135 ethnically matched healthy controls for allelic determinants at two polymorphic sites, one in the promoter region and another in the first exon at position +49 by MseI and BstEII restriction enzyme digest analyses, correspondingly.
Allele frequencies of C-318T gene polymorphism in patients and in healthy controls for allele C were found to be 86,74% and 88,15% correspondingly. The frequency of the allele A of A+49G

polymorphism was 48,91% in the MG group compared with 50,0% in the control group. There was no significant difference in the allele frequencies distribution of these loci between patients and healthy controls. But when the patients were the stratified according to their thymus histopathology, patients with thymoma had more often allele T in the promoter region position - 318 of CTLA4 gene in compare with the healthy control and patients with thymic hyperplasia that might confirm the pathogenetic geterogeneity of MG. Our results did not show any evidence of association of C-318T and A+49G of CTLA4 gene polymorphisms with MG.

P0982. Among the males bearing the MTHFR 677T allele, the normotensive persons have the greater risk of abdominal aortic aneurism (AAA) as compared to the hypertensive persons.

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Interaction between the arterial hypertension and development of the abdominal aortic aneurism (AAA) has been assessed in a group of 62 patients with AAA. Significantly higher frequency of persons with the MTHFR 677 TT and CT genotypes has been noted in the group of persons with normal arterial pressure (14/19) as compared to that in the group with arterial hypertension (13/31) (p=0,03; OR=3; 95% CI 0,9-10). In the group of smoking patients with normal blood pressure the MTHFR 677 TT and CT genotypes occurred at even higher frequency (9/12) as compared to the frequency in the group of nonsmoking patients with high blood pressure (3/10) (p=0,02; OR=7; 95% CI (1,7-45,9)). The group of smoking AAA patients displayed also the significantly higher frequency of MTHFR 677T allele, as compared to the nonsmoking AAA patients (0,36 vs. 0,21; p=0,03). The highest frequency of MTHFR 677T allele (0,42) was noted in the group of smoking AAA patients with normal blood pressure. These observations seem to indicate a distinct pathomechanism of AAA development in the normotensive persons.

P0983. The analysis of association between polymorphism of BGLAP gene and gynecologic diseases at osteoporosis in postmenopausal women

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Osteoporosis is a common multifactorial disease with a strong genetic component. One of the important factors of pathogenesis of osteoporosis in postmenopausal women can be such gynaecology disease as endometriosis and mioma. Nowadays a number of genes have been identified, which play important role in bone metabolism and reproductive function of women. For several genes exact mutations were discovered, which were shown to result in activity changes of corresponding proteins.

The polymorphism BGLAP (osteocalcin) gene was studied by PCR-RFLP method. The RFLPs were represented as **H** or **h** (*Hind* III) for BGLAP gene. The allele of BGLAP gene in 64 non-related individuals without osteoporosis rates Northwest Russian population (control group) and in 40 women with normal menopause (1 group) and 57 women with surgical menopause (2 group) were investigated. In this group of women the frequency of osteoporosis with endometriosis and mioma was rather high (more than 80% of all patients).

The frequency of functionally abnormal allele **H** in-group 2 was 23,7%, which is significantly higher (p<0,01) than only in group 1 (10,0%). The frequency of this allele in control group was 18,1% and did not significantly differ from the average in group 1 and group 2 (p>0,05).

The present study suggests that the presence of the **H** allele of BGLAP gene is predictive factor of gynaecology diseases in women with surgical menopause. The testing for alleles of BGLAP gene is considered to be perspective for prediction of the development of gynaecology diseases and selection of optimal therapy of osteoporosis.

P0984. Interleukin-4 receptor alpha chain polymorphism and atopic dermatitis

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Atopic dermatitis and other atopic diseases have a strong genetic predisposition. Mutations in the subunit of the IL-4 receptor in patients diagnosed with atopic dermatitis have been reported to be more frequent than in healthy controls. However, apparently the association differs among ethnic groups. Therefore, we screened the whole Il-4 receptor gene from genomic DNA of 11 patients with atopic dermatitis from our outpatient clinic. Subjects with atopic dermatitis were identified on the basis of clinical histories, positive RAST and elevated total IgE level. Eight amino acid substitutions have been found: one substitution in the extracellular domain and the remaining 7 in the intracellular domain. Six probands showed one or more mutations in the IL- 4 receptor alpha chain (6 the Q576R polymorphism, 2 the C431R, 1 the I75V and S436L). The number of mutations did correlate with higher levels of total IgE, whereas patients with low IgE levels tended to have no mutations. This is in contrast to the theory that patients with intrinsic atopic dermatitis (featuring low levels of total IgE) are supposed to have strong genetic background. In addition, we could not find an association with lower total IgE levels for the Q576R polymorphism, because 5 of 6 patients with this polymorphism showed a total IgE of more than 1000 kU/l. We conclude that a high percentage (54,5%) of patients with atopic dermatitis in the Salzburg population shows polymorphisms in the IL-4 receptor alpha chain. Especially, the Q576R allele is very common (6 of 11).

P0985. Mapping of a novel locus for essential hypertension in an isolated population in Germany

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Isolated populations might feature considerably fewer numbers of genetic variants compared to heterogeneous populations, thus increasing the power of finding genes relevant to complex traits. In a study on essential hypertension (n=326), the population of interest are the Sorbs, a putative population isolate in Germany. We aimed (1) to evaluate the genetic diversity of the Sorbs, and (2) to search for oligogenic configurations of markers contributing to hypertension. Methods: We tested the genetic homogeneity of the Sorb population and the hypertension phenotype by separate multivariate feature vector analyses (genetic vector space). For measuring genetic homogeneity and for detection of population substructure we established a set of 18 well-defined autosomal microsatellite markers (ethnicity panel). For genotype-phenotype analysis of hypertension we performed a genome-wide scan and chose 400 evenly distributed microsatellite markers for this tentative analysis.
Results: We found a significant reduction of genetic diversity in our study population. This confirms, that the Sorbs have a reduced genetic heterogeneity and actually are an isolated or founder population. We could also genetically identify two population subgroups in agreement with slightly different population histories. Tentative multivariate feature vector analysis with respect to hypertension identified a genomic region on chromosome 11q significantly associated with a vulnerability factor of hypertension. This locus is consistently significant in both subgroups and in the pooled sample. Our investigation confirms the power of isolated populations in mapping genes for complex traits and promises to identify new loci responsible for arterial hypertension.

P0986. Prevalence of microdeletions of the Y chromosome in Tunisian infertile men with idiopathic azoospermia and oligospermia

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Recent investigations have pointed to a high prevalence of Y chromosome sub microscopic deletion in men with severe impaired spermatogenesis. We report on the incidence in 146 Infertile men, in whom karyotype and sperm count were evaluated. Patients with abnormal karyotype were excluded.
Genomic DNA was extracted from peripheral leukocytes of 76 men with azoospermia, and 70 men with oligospermia. Molecular analysis was performed on 2 steps according to the French Society of human genetics and The European Association of Andrology recommendations.
In 6.84% of the studied patients, Yq microdeletion were detected with a prevalence of 11,84%(9/76) in men with azoospermia and 1.43% (1/70) in men with oligospermia. . 3 Of our patients had deletion of the three AZF regions, No deletion were detected in AZFa region only , 3 other had deletion of AZFb and AZFc regions , 3 patients with AZFc region only and 1 patient with AZFb region only. No deletion were detected in AZFa region only. Because men with severe infertility suffer a high risk of chromosome deletion, screening of these men is recommended prior to treatment with assisted reproduction.

P0987. Gene polymorphisms of CC16 and NOS1 in pregnant women with asthma

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The genetic polymorphisms of two genes: NOS1, CC16 were studied by PCR-RFLP analysis in groups of Russian pregnant women with atopic bronchial asthma (ABA) (60 individuals, 25,8±3 years) and healthy female (50). In view of chromosomal location and functional role in respiratory inflammatory processes of studied genes, abnormalities of the CC16 gene (12q24) or the NOS1 gene (11q13) may be involved in inherited pathogenesis of asthma. Asthmatics had lower mean plasma CC16 levels than nonasthmatic subjects. Also an increased concentration of nitric oxide in exhaled air is now recognized as a critical component of the asthmatic phenotype. Increased concentrations of NO in exhaled air are closely related to the number of (AAT)(n)-repeats in intron 20 of NOS1 gene. The distribution of genotypes and alleles of the A38G polymorphism in the CC16 gene were not significantly different between patients and control groups. On the other hand, we observed the 2-fold increasing of the frequency of individuals with a high number (>=12) of AAT repeats of the NOS1 gene in control group. These genotypes are associated with lower level of nitric oxide in exhaled air. However this difference wasn't significant (p=0,07) but perhaps an increase of the number of individuals in studied groups will allow to detect a significant difference.The results suggest that the A38G polymorphism in the CC16 gene is not associated with atopic bronchial asthma in Russian population.

P0988. A functional SNP in Alcohol Dehydrogenase 3 Modulates the Effect of Alcohol Consumption on C-Reactive Protein: Results from a Large Population-Based Study.

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A polymorphism in the gene coding for alcohol dehydrogenase type 3 (ADH3) alters the rate of alcohol metabolism and might modify the beneficial effects of moderate alcohol consumption on coronary heart disease (CHD). C-reactive protein (CRP) represents a strong and independent predictor of coronary events. We investigated the effect of alcohol consumption on levels of CRP and HDL cholesterol and its relation to ADH3 genotypes. The ADH3 genotypes (γ1γ1, γ1γ2, or γ2γ2) were determined in 1806 men and 1844 women of the third MONICA-Augsburg survey (1994/95) using MALDI-TOF MS analysis (Sequenom inc.). Adjusted mean levels of CRP and HDL cholesterol were calculated in defined categories of alcohol intake and related to the ADH3 genotypes. Daily alcohol intake showed an apparent J-shaped association with CRP concentration in both sexes. In all ADH3 genotype groups CRP levels were lower among those who consumed >0-40 grams daily compared to non-drinkers. Among the homozygotes for the γ1 allele and the heterozygotes, CRP concentrations raised with increasing alcohol consumption. However, among those homozygous for the γ2 allele, CRP concentrations showed a further decrease among consumers of >40 grams daily. CRP concentrations among the various genotypes in this group showed a statistically significant difference (p=0.02). In all ADH3 genotypes, HDL concentrations showed a similar significant increase with increasing alcohol intake (p<0.0001). Non-drinkers and heavy alcohol drinkers had higher CRP concentrations than moderate drinkers. Homozygosity for the γ2 allele of the ADH3 gene was associated with the largest decrease of CRP levels.

P0989. The search for molecular-genetic risk markers of suicidal behavior in two differen ethnic groups from Bashkortostan, Russia

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Suicide is an important public - health problem; in Russia suicide rate is in excess of 39,7 per 100000 inhabitants per year. Risk factors for suicide are composed of genetic and environmental influences. Patients with different psychiatric disorders are a group of higher risk for suicide attempts. A serious problem of the suicide prediction in patients from Bashkortostan is their different ethnic origins. As it is known there are differences in genotype and allele frequency distributions of candidate neurotransmitter systems genes between different ethnic groups.
The aim of the present study was to value of eight candidate genes contribution in suicidal behavior in patients from Russian population which belongs to Slavic group of Indo-European language family and Tatar population which belongs to Turkic group of Altaic language family. 197 suicide attempters of Russian (112) and Tatar (85) descents and 301 control subjects of the same ethnic background were typed for the SLC6A4, HTR2A, HTR1B, SLC6A3, DRD2, MAOA, COMT, TPH gene variants using PCR technique.
According to our data different candidate genes can be associated with suicidal behavior in patients of Russian and Tatar descents. For the Russians the HTR1B G/G (OR=2.26, 95%CI=1.30-3.91), the SLC6A4 L/L 12/10 haplotype (OR=2.11, 95%CI=0.94-4.77), the TPH A/A (OR=2.41, 95%CI=1.00-6.00) and the COMT H/H (OR=2.11, 95%CI=1.01-4.44) are possible risk markers of suicide attempts. For the Tatars the HTR1B C/C (OR=0.29, 95%CI =0.09-0.89) and the HTR2A G/G (OR = 0.54, 95%CI = 0.27-1.07) are protective markers, the COMT H/H (OR=2.70, 95%CI=1.33-5.51) is a risk marker.

P0990. Schooling as a complex genetic trait in an urban underprivileged group

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Fight against poverty in developing countries is envisaged through several disciplines that unfortunately rule out intervention of genetics, thus neglecting its importance on every human trait. Schooling could be considered as a genetic complex trait (CT), dependent on behaviour, and modulated by the environment. We hereby present a first attempt to analyse Schooling considering it as a semi-quantitative and CT, through segregation analysis (SA). A collaborative project (SPV/UNR), enabled us to carry on the study in a shanty town (ST: 270 pedigrees; n=1264 individuals). ST random sample: 25 pedigrees, n=247 (parents: males=70, females=64; full-sibs=86; half-sibs=11; grandparents=16). Schooling and socio-economical indicators were collected applying a voluntary extended questionnaire. Schooling level was scored with values ranging from 10 to 80, taking into account our educational programs, starting at 6yr of age. Data process: PEDINFO and FCOR modules (SAGE 4.3), and MAN for SA were used. Schooling was present only in 50% of the sample. Males (6-64yr) Schooling-score 25±8. Females

(6-68yr) 27±8. Heritability (r): Parent/offspring: 0.42; siblings: 0.48. SA rejects a Mendelian transmission model. Schooling-score in this sample is remarkable low. Since our first results point out a non-major gene model, the trait might be improved changing economic-social- psychological conditions. This implies not only offering passive solutions but encouraging the participation through positive behaviour (e.g. Schooling) of the target individual/population, as well as community commitment of all citizens and specialized professionals to implement solutions conjointly. Poverty-wealth is a continuum in human beings, solidly linked to biological traits more or less dependent on environment.

P0991. Blood pressure (BP) heritability in an urban underprivileged group. The right to genetic health knowledge.

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Genetic complex diseases prevention is not fully understood and/or accepted in developing countries, least of all sufficiently spread in public health policy implementation, since genetics is considered an elite medical specialty to which underprivileged groups have no access in spite of the WHO health definition. A collaborative project (SPV/UNR), enabled us to carry on a study on a shanty town (ST: 270 pedigrees; n=1264 individuals). Reference values: from an urban cohort (UC) with middle-high socio-economic status. Both groups share similar ethnical features. ST random sample: 25 pedigrees, n=247 (parents: males=70, females=64; full-sibs=86; half-sibs=11; grandparents=16). Voluntary extended questionnaire, several anthropometric measures, and systolic BP (SBP), diastolic BP (DBP) (recommendations: V-JNC-1993) were obtained. Data process: PEDINFO and FCOR modules (SAGE 4.3). Age: Males =>18yr, 38% of the sample, aged 30±12yr, cumulative % 70 (27yr); some variables: BMI 23±4; SBP 119±12mmHg; DBP 74±7mmHg. Females =>18yr, 55%, 29±12yr, cumulative % 73 (29yr); BMI 26±7; SBP 110±11; DBP 72±8. Familiar correlations (r±SE): Table. 1) ST population is very young with values same to the reference ones (not shown), except for lower male BMI. 2) half-sibs were detected in ST; 3) r-parent/offspring: SBP and DBP similar in both groups; 4) similar DBP r-siblings, while negative for SBP in ST, and low in UC. 5) results lead us to remark the importance of environment, family constitution, etc, in general and genetic health policies implementation, as well as strongly support the unconditional right to genetic health knowledge access to all individuals in spite of their social-economical status.

	ST			UC		
	Par-Off H2 ±; SE	sib-sib H2±;SE	Half sibs H2±;SE	Par-Off H2±;SE	sib-sib H2±;SE	Half sibs H2±;SE
SBP	0.28±;0.10	-0.11±;0.11	-0.39±;0.30	0.26±;0.04	0.05±;0.09	
DBP	0.11±;0.15	0.24±;0.19	0.30±;0.41	0.18±;0.04	0.30±;0.09	

P0992. Geography of surnames in Azores: specificity and spatial distribution analysis

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Geographic isolation and discontinuity are important factors to be considered in genetic studies of populations living in islands. To obtain a better understanding of the genetic structure of Azorean population, a specificity and spatial distribution analysis was performed based on 2454 different surnames present in the Azorean telephone directory (2002).
Specificity analysis is a useful method to characterize surname diversity. We considered as specific surnames those with frequency equal or higher than 50%. The results revealed 33 specific surnames in 12 of the existing 19 municipalities. The smallest municipality presents the only surname with 100% of specificity (Pedras). In addition, Terceira island with 2 municipalities shows 6 specific surnames (Lourenço, Toste, Barcelos, Meneses, Valadao and Areias). Overall, the data suggest that specific surnames may be regarded as genetic markers.
The spatial distribution analysis was used to detect genetic similarity between municipalities through the calculation of spatial autocorrelation (Moran's *I*). Of the 240 surnames studied, 113 showed statistically significant patterns. We obtained 5 different patterns and the most relevant is "isolation by distance and depression" (41.6%). However, 43.4% had no defined pattern. The overall correlogram shows a majority of positive values (surname similarity) for distances <49km and between 269-309km. This demonstrates high similarity between closer municipalities and between distant municipalities whose populations show historic and socio-cultural affinities.
Although the Azores may constitute a genetically homogeneous isolate, a better knowledge of its population structure will contribute to understand the patterns of the inherited diseases in this archipelago.
Funded by DRCT (Azores). rtrrc@lycos.com

P0993. Genetic And Epidemiologic Study Of Congenital Eye Malformations In 293,923 Consecutive Births

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Congenital eye malformations(CEM) were studied in 293,923 consecutive births during the period 1979-2000.The prevalence rate of CEM was 6.8 per 10,000, for microphthalmia1.7,anophthal mia 0.23,cataract 2.7 and coloboma 1.4,respectively.Sex ratio was 0,79.Prenatal diagnosis was performed in 27 cases and 12 cases were induced abortions.The more common types of associated malformations in the 108 affected cases(53.2%)with at least one anomaly other than an eye malformation were clubfeet,microcephal y,hydrocephaly,cleft lip/palate and facial dysmorphia.At birth infants with CEM and other malformations were smaller,weighted less and their head circumference was lower than in controls.Placental weight was also lower than in controls.Prenancies with CEM were more often complicated by threatened abortion,oligoamnios,and polyhydramnios.Mothers of children with CEM took more often drugs during pregnancy than mothers of controls.Fathers of children with CEM were more often exposed to occupational hazards than fathers of controls.
There was a significant association between eye malformations and consanguinity of parents.The recurrence risk for first degree relatives of probands was 8.7%.First degree relatives of probands had more than three times the prevalence of non-eye malformations than controls.
These results are of relevance to genetic counseling.

P0994. Epidemiological study of congenital malformations in Gorno-Altaisk (Altai Republic)

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The results of retrospective epidemiological study using materials of medical clinics in Gorno-Altaisk since 1983 to 2001 years have been analyzed. The most frequent malformations were of the osteomuscular system (37,68%), cardiovascular system (18,22%) and multiple congenital malformations (8,9%). The frequency malformations of osteomuscular system is 7,38‰, cardiovascular system - 3,57‰ and multiple congenital malformations -1,74‰ per 1000 birth. Congenital malformations have been found among newborns, in children who died before 1 year of age and in fetuses after 22 weeks gestation. In Russia birth defects are monitored according to a system that contains 21 nosological forms. The incidence of multiple congenital malformations was 6, 08 per 1000 birth. The frequency of Down syndrome was 0,93 per 1000 births and did not show any statistically significant changes during the study period.

P0995. Human DNA bank in São Miguel Island, Azores: Assembly and analysis

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The establishment of a human DNA bank is of particular importance in the characterization of the population's polymorphisms, particularly in geographically isolated places.
Here we describe an ongoing project to build a DNA bank in São Miguel, the biggest and most populated island of the Azores, and we analyse the statistics of the first 750 samples (December 2002 to January 2004). The DNA bank will consist of 1,000 healthy and unrelated individuals (0.8% of the current population), whose samples are obtained in collaboration with the Haematology Department.
The bank follows the international ethical guidelines, which include Informed Consent, confidentiality and anonymity of personal data, and abandonment of the study in case of expressed will. DNA is isolated from blood samples (7.5 mL), coded and immediately stored in a locked refrigerator.
The identifiable DNA bank described here has self-reported data concerning sex, age, birth, current place of living, and parental birthplaces. All samples collected to date are representative of all the island's municipalities ($r=0.987$, $p<0.01$). The majority (86%) of the participants are male, and the mean age is 35.7 y (18-63y). Birthplace analysis reveals that 660 (87%) have both parents born in São Miguel. Moreover, 389 (59%) have their parents born in the same locality, confirming high rate of consanguinity in rural area.This DNA bank is of strategic importance for genetic research and for the better understanding of the genetic structure of the Azorean population. Funded by DRCT, Azores.

P0996. The prevalence of hereditary pathology of the populations Altai Republic

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The results of medical-genetics investigation population Altai Republic are represented in this article. The number of investigative population was 203148 people, including 59196 Altais, 134972 Russians, and 8980 Kazakhs. The load Mendelian pathology with different types of heredity was determined for each ethnic group, taking into account the territorial distribution: town, village. The 36 nosological forms of autosomal dominant diseases with 121 patients were revealed, and more often was the group of hereditary syndromes. Autosomal recessive pathology includes 24 nosological forms with 158 patients. The group of metabolism defects predominates. X-linked diseases are represented 4 nosological forms, 9 affected subjects were found. Prevalence was estimated for all diseases in the regions where they were found. In the urban population, the load of autosomal dominant, autosomal recessive, X-linked pathology were: Altains - 2.98; 9.62 per 1000 individuals respectively, and 0.56 per 1000 male; Russians - 0.86; 0.94 per 1000 individuals respectively, and 0.23 per 1000 men; Kazakhs - 0,34; 1,16 per 1000 individuals respectively. No one case of X-linked pathology was found in Kazakhs. The load of autosomal dominant, autosomal recessive, X-linked pathology was estimated in the rural population for isolated regions. The hereditary pathology spectrum in the populations studied was described.

P0997. Distribution the VNTR alleles at the PAH gene in population of Moldova.

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Phenylketonuria is an autosomal recessive genetic disorder caused by phenylalanine hydroxylase (PAH) deficiency. The PAH gene has been cloned and mapped on Cr. 12 position 22-24.1 and consist of 13 exons. The final exon contain various number of 30bp tandem repeats (VNTR) and previously reported as Hind III polymorphism in the PAH gene (Woo et al., 1983).
Genomic DNA was extracted and examined by standard procedures from 197 members of PKU families. VNTR analysis was performed as described by Goltsov et al. (1992).
Allele frequencies were established by examining of 59 patients with phehylketonuria and 67 members from health population. The most prevalent among mutant and normal chromosomes was VNTR allele containing tree and eight repeats (67,8% and 42,7%, respectively). The two most common alleles, the 380bp and 530bp, together account for about 75% of normal and 87% of mutant chromosomes. The VNTR allele containing seven repeats is the third most frequent allele among both normal and mutant chromosomes examined in this study. Several alleles (470bp and 650bp) were absent from PKU patients. Only one normal allele bearing six VNTR units was observed. VNTR alleles had a significant difference in the distribution among normal and mutant chromosomes ($\chi^2 = 32,2$; $p<0,001$). The average level of
heterozygosity of the VNTR system in population of Moldova is 71,2%. Frequency of informative cases by VNTR analysis from PKU families is 48,8 percent. The high degree of polymorphism of VNTR system suggested useful for prenatal diagnosis and carrier determination in PKU families in Moldova.

P0998. Allele and genotype frequencies of polymorphic cytochrome P4502D6 in a Spanish population

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CYP2D6, which metabolizes 25-30 % of medications used, is characterized by a broad interindividual and interethnic variability in its activity. Multiple mutations confer divergent functional significance, ranging from a total loss of activity (gene deletions and splicing or frameshift mutations) to an increase in enzyme activity (gene amplifications). Moreover, different point mutations are associated with reduced CYP2D6 activity.
Individuals can be classified in different phenotypes in accordance with the activity of the enzyme. Poor Metabolizers (PM) are those that lack CYP2D6 activity and have non-functional alleles (*3, *4, *5, *6, *7, *8, *12, *14, *15 and *21). Intermediate Metabolizers (IM) that may be either heterozygous for one inactivating mutation or homozygous for allele associated with impaired metabolism (*9, *10). Ultrarapid metabolizers (UM) are those who carry additional copies of the CYP2D6 gene and Extensive metabolizers (EM) carry wild-type alleles.
Genotyping for 15 CYP2D6 alleles was performed in 105 volunteers. The most frequent alleles were: *2 (40.47%), *1 (31%), and the PM allele *4 (13.8%). Two IM alleles *9 and *10 were found with frequencies of 2.38% and 1.90% respectively, whereas the PM allele frequencies were 0.95% (*3 and *6) and 3.33% (*5). Other inactive alleles (CYP2D6 *7, *8, *12, *14, *15 and *21) were not detected in any subject of this study. The frequency of gene duplications in this population was 8.57%.
While the frequencies of alleles with single-base changes resemble to those found in other Caucasian populations, alleles with gene duplications are found at a significantly higher frequency.

P0999. Genetic study of susceptibility to tuberculosis in Tuvinians

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Tuva is the region of Russia with the highest prevalence of tuberculosis (TB) at all times. We hypothesized that this situation occurred because of a unique genetic susceptibility of Tuvinians. To test it we studied common polymorphisms of the human *NRAMP1* (D543N, 469+14G/C, C247T, 1465-85G/A), *IL12B* (1188A/C), and *VDR* (F/f, B/b) genes in Tuvinians with TB (n=100) and without this disease (n=263) and also in healthy Russians inhabitants of Tomsk (n=127).
A high ethnic specificity in prevalence of alleles of the TB candidate genes studied was found: the prevalence of the D543 and 1188A alleles was higher in Tuvinians and the prevalence of 469+14G,

1465-85G, and C274 was higher in Russians (p<0.05 in all cases). There was no significant association between TB and any of the polymorphisms investigated. However, there was a high prevalence of genotype b/b of *VDR* gene both in affected and healthy Tuvinians (~66 %). This allele b is in strong linkage disequilibrium with the T allele of the same gene. It is known that allele T has a pathological meaning for TB. We suggest that the higher susceptibility to TB in Tuvinian people in comparison with others is based on the increased frequency of allele b(T) in this population.
On the whole, the data obtained support the necessity for a further investigation of TB susceptibility candidate genes in an expanded sample of Tuvinians.
This work was supported partially by Russian President's grants (Leading scientific schools-840.2003.4 and Young scientists-742.2003.04).

P1000. Mutation scanning of *LDLR* in the whole population: severe, moderate and silent mutations, paucimorphisms and cholesterol level

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We have developed and applied to *LDLR*, a mutation scanning approach suitable for whole population screening for unknown mutations. Applications include definition of population-based ‚reference ranges' for rarer sequence variation; characterisation of ‚paucimorphisms' (arbitrarily defined here as variants of rarer allele frequency, 0.05%<q<5%); research of contribution of ‚formes frustes' milder mutations that are of significant relevance to the individual; and identification of severe mutations at the population level. The method, meltMADGE, is a reconfiguration of DGGE (using a thermal ramp rather than a urea gradient) enabling combination with microplate array diagonal gels (MADGE). Throughput per day per person is 4x10x96well gels in 2x2l tanks, representing 4,000 amplicons. Scanning cost: 50Eu/Mb; rate 10Mb/week.Assays of *LDLR* exons 3 and 8 were validated in 460 familial hypercholesterolaemics with known mutations. We then applied the exon 3 assay in several DNA banks representing ~9,000 subjects with known cholesterol values and applied both assays in one DNA bank (n=3,600). In exon 3 we identified one known forme fruste mutation, P84S (n=1), also associated with moderate hypercholesterolaemia in this subject; an unknown silent variant, N76N (n=1); and known severe hypercholesterolemia splice mutation 313+1 G>A (n=2). Around exon 8 we identified a paucimorphism (n=35) at splice site 1061-8 T>C (known to be in complete linkage disequilibrium with T705I); and unknown splice 1186+11 G>A (n=1) and D335N G>A (n=1). D335N and a significant fraction of T705I subjects displayed cholesterol values above the 95th centile. Thus both severe, moderate and silent variants were identified, at the population level.

P1001. Human Y-chromosome haplogroup E3b in Africa: a phylogeographic study

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We explored the phylogeography of human Y chromosomal haplogroup E3b by analyzing 677 individuals from different African regions. An estimate of 25.6 ky (95% CI: 24.3-27.4 ky) for the TMRCA was obtained for the haplogroup E3b, which most likely originated in Eastern Africa. Our data refine the phylogeny of the entire haplogroup, which appears as a collection of lineages with very different evolutionary histories. The distribution of E-M81 chromosomes in Africa closely matches the present area of distribution of Berber-speaking populations in the continent, suggesting a close haplogroup-ethnic group parallelism. E-M34 chromosomes were more likely introduced in Ethiopia from the Near East. Haplogroup E-M78 was observed over a wide area, including eastern (21.5%) and northern (18.5%) Africa. A microsatellite-based network of the E-M78 chromosomes revealed a strong geographic structuring, with two well differentiated sub-clusters, one of which being present exclusively in eastern Africa and the other in northern Africa. A new biallelic marker (V6) was discovered in the present survey in the TBL1Y gene by DHPLC analysis. This marker identifies a subset of chromosomes (haplogroup E-V6) previously assigned to E-M35*. The haplogroup E-V6 was only observed in Eastern Africa (8.9% in Ethiopia, with a single occurrence both in Somalia and Kenya), further testifying to the richness of E3b lineages in this region.

P1002. The Y chromosomal heritage of São Miguel's population (Azores)

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The island of São Miguel had no native population when the Portuguese first arrived in the XV century. The island was populated mainly by Portuguese but Jews, Moorish prisoners, African slaves, Flemish and Spaniards also contributed to the initial settlement.
To understand the paternal origins and diversity of extant São Miguel's population we typed genomic DNA samples from 197 individuals, of which 149 from São Miguel Island, 23 from other Azorean islands and 25 from mainland Portugal. A total of 10 biallelic markers (YAP, SRY-1532, SRY-2627, 92R7, M9, sY81, Tat, SRY-8299, 12f2 and LLY22g) were used and the following Y–chromosomal STR systems DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385.
We identified 8, 7 and 5 distinct haplogroups (HG) in São Miguel, mainland and other Azores islands, respectively. HG 1 is the prevailing among all individuals, with the highest frequency (60.4%) in São Miguel. The second most common HG is HG9, with the highest frequency of 26.1% in other Azorean islands. Worthy of note is the presence of HG8 (1.3%) and HG16 (0.7%) only in São Miguel Island. The combination of variation of the STR markers gave rise to 137 haplotypes out of 197 samples, none of which was shared by all three groups. The two most frequent haplotypes are 13-17-24-11-13-13-11/14 and 13-17-24-10-13-13-11/14, both with a frequency of 5%. The data shows the presence of African and North East European haplogroups, however the current paternal Y-chromosome pool in Azores is, to a great extent, of Portuguese descent. Funded by DRCT-Açores.

P1003. Iranian Human Mutation Gene Bank

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The data base of Iranian Human Mutation Gene Bank is a comprehensive source of information on DNA samples collected in this bank during the last 6 years. The samples belong to the patients with genetic disorders with mendelian mood of inheritance studied in Iran. Some of the samples are assigned to common 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 categories of genetic disorders including Hemoglobinopathes , Neuromuscular disorders, Mental Retardations and Hearing loss. with the exception of personal data, which is strictly kept confidential, clinical profile for each individual, and genetic data including pedigree for each family is presented in this data base. 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 providing opportunities for us to collaborate with outside, will offer a free of charge sample resource from a large heterogeneous population to all the scientists in the world, who are working on the various aspects of genetic disorders from prenatal diagnosis to gene structure and function. No commercial benefit is involved in establishment of this DNA bank and the DNA samples are free of charge. Please visit our under construction website on http://www.IHMGB.com (Its link is also available in HUGO official website).

P1004. Genetic differentiation of the population of Northern Asia Inferred from Alu-insertions.

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We have described the genetic differentiation of North Asia populations using eight polymorphic Alu loci. These are seven polymorphic Alu insertions (ACE, PLAT, APOA1, PV92, F13B, A25, D1) and CD4 loci. Locus CD4 is characterized by deletion 256-bp from 285-bp Alu-element, i.e. unlike other Alu-repeats the presence of insertion is original. We have studied a total of 2383 unrelated individuals belonging to 34 populations from 19 ethnic groups of Northern Asia. Studied ethnic groups belong to two basic racial types of Eurasian population (Caucasoid and Mongoloid) and speak the languages of six linguistic families – Altai, Indo-European, Ural, Chukchi-Kamchatka, Eskimo-Aleutian and Sino-Tibetian. All eight loci proved to be polymorphic in all populations examined: no case of allele fixation was found. CD4 locus exhibited an interesting feature: frequency of Alu (-) clearly decreased with increased „Mongoloidity“ in the population. In the studied populations from Siberia, Central Asia and the Far East the lowest frequency was observed for Eskimos (0.012) where Mongoloid component was strongly pronounced and the highest frequency was observed for Russians and Ukrainians (0.35). Gene pool of the Northern Eurasian populations representing different geographic regions and linguistic families was found to be highly differentiated: G_{ST} value accounted for 7.5 %. Such a degree of between-population genetic differences completely corresponds to the complexity of the concerned ethno-population system.

P1005. Interactions between genetic polymorphisms and antihypertensive drugs in the treatment of hypertension

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Despite the availability of a variety of effective antihypertensive drugs, inadequate control of blood pressure is common in hypertensive patients, and responsible for a large proportion of the burden of myocardial infarction (MI) and stroke in the population. Identification of genetic polymorphisms which modify the response to antihypertensive drugs provides the opportunity to optimise safety and effectiveness of the currently available antihypertensive drugs. The primary objective of the study is to assess whether reduction of MI risk is larger in diuretic users who have the AGT-235T, the alpha-adducin-460T, the Gp-beta-825T, the ACE-I, or the AT1R-1166C alleles than in diuretic users who are homozygous for the wildtype alleles.
In a population-based registry of pharmacy records linked to hospital discharge records (PHARMO), we will use a nested case-control design to assess whether these polymorphisms modify the effect of antihypertensive drugs on the risk of MI. Cases and controls will be recruited through community pharmacies and are asked to fill in a questionnaire and supply a sample of buccal cells for DNA-genotyping. Logistic regression analyses will be used to assess antihypertensive drug-gene interactions and to adjust for potential confounding factors.
In total, 1379 MI cases were selected from the PHARMO database. To each case 9 controls were matched on age, gender and pharmacy.
So far, 350 cases and 751 controls were approached and we have reached a response of 37,3%.Understanding the association between antihypertensive drug-gene interactions and various cardiovascular outcomes may eventually help physicians to tailor drug therapy to the individual patient.

P1006. Preliminary estimation of COX-deficient Leigh syndrome carrier frequency in Polish population

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Leigh syndrome (LS), (MIM 256000) is a progressive neurodegenerative disorder of infancy or childhood with characteristic pathological hallmarks presented as symmetric necrotizing lesions in the brainstem, basal ganglia, thalamus and spinal cord. Clinical presentation includes muscle hypotonia, developmental delay, psychomotor retardation, respiratory abnormalities, brainstem or basal ganglia dysfunction. A typical biological feature is hyperlactatemia. LS results from several defects of mitochondrial oxidative phosphorylation, the most common involving pyruvate dehydrogenase complex, NADH-ubiquinone oxidoreductase (complex I), and cytochrome c oxidase (complex IV, COX). Mutations in the COX assembly gene *SURF1* are responsible for most of the cases of Leigh syndrome with cytochrome c oxidase deficiency.
Our earlier study performed on 31 Polish COX deficient Leigh patients revealed the presence of 5 different mutations: 312insATdel10, 758delCA, 845delCT, M235T, Y274C.
Deletion 845delCT was identified on 81% of mutated alleles. High prevalence of one common mutation let us to choose it as a marker of LS carriers in Polish population. The aim of our study was to establish the frequency of heterozygote LS carriers. Up to now 1660 DNA samples, collected on anonimous Guthrie cards , have been screened. Five heterozygotes for 845delCT mutation were detected (0,30 ± 0,26%). A tendency for clustering them in two old, essential Polish areas was observed. Preliminary estimated theoretical frequency of COX- deficient Leigh syndrome in Polish population is about 3,4 in 1000000 births.
The study was partly supported by the State Committee for Scientific Research in Poland, Grants no. 3 PO5E 095 25 and no. S76/2000.

P1007. Identification of mitochondrial DNA haplogroups in a population sample of central Italy

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The two hypervariable regions (HVR I and HVR II) and haplogroup-specific coding region sites of mitochondrial DNA were analyzed in a population sample from Central Italy, in order to evaluate mtDNA variability and haplotype distribution. 77 DNA samples were submitted to direct sequencing of the D-loop region (HV1-nt15971-16414; HV2-nt15-389) and to enzymatic restriction fragment-length polymorphism (RFLP) analysis of the coding region by PCR amplification of eight fragments, followed by digestion with 7 restriction endonucleases (AccI, AluI, DdeI, HaeIII, HinfI, MseI, NlaIII). Analysis of the two hypervariable regions allowed detection of 70 different haplotypes. By means of combination of haplogroup-specific restriction site changes and control region nucleotide substitutions, these samples were classifed into well-defined haplogroups. Most of the mtDNA haplotypes detected in Italians fall into the common West Eurasian mitochondrial haplogroups and their subgroups: H (37.7%), pre-*V1(2.6%), pre-HV (1.3%), HV (11.7%), V (2.6%), U (9 %), K (6.5%), J (7.8%), T (13%), X (5.2%), W (1.3%). In addition, two East Eurasia specific sequences, belonging to haplogroup D, were observed.

P1008. Development of six multiplex PCR with 37 Y-chromosome SNPs

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Their low mutation rate, patrilineal inheritance, and absence of recombination make Y-SNPs particularly suitable for evolutionary studies. Many SNPs also show regional specificity, providing useful information about human evolution, population genetics, and allowing reconstruction of family relationships by patrilineage analysis. Lastly, amplification of short fragments, including the single base mutation which characterizes their polymorphism, means that they can be used in forensic genetics, particularly in cases of highly degraded DNA.

The aim of this study was to set up multiplex PCRs of NRY SNPs suitable for forensic purposes and evolutionary studies. Markers were drawn following a hierarchical strategy based on the phylogenetic tree proposed by the Y Chromosome Consortium (YCC), which unifies the several unrelated and non-systematic previous nomenclatures and classifies the major clades of the tree into 18 haplogroups, indicated by capital letters from A to R.

Two multiplexes were developed to explore the basal branches of the tree: MY1 (M35, M89, M172, M170, M9, M173, M45) and MY2 (M52, M216, M174, M181, M201, M91, M96, M214). Four multiplexes for the more superficial branches were also developed: MY-E3b (M78, M107, M224, M165, M148, M81), MY-J2 (M158, M68, M47, M12, M137, M67), MY-R1 (M17, M269, M18, P25, SRY10831.2) and MY-I (M72, M223, M26, M21, M161).

SNP genotyping was carried out by hot-start PCR with primers amplifying fragments between 63 and 171 nucleotides, with minisequencing based on dideoxy single-base extension of unlabeled oligonucleotide primers using the SNaPshot multiplex kit (Applied Biosystems) and capillary electrophoresis of extension products.

P1009. Heritability of pulse wave velocity in an extended pedigree from an isolated population

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Object: Pulse Wave Velocity (PWV) is a measure of arterial stiffness and has been associated with cardiovascular diseases. There is increasing interest in the genetics of arterial stiffness but little is known of its heritability. We studied the heritability of PWV in an isolated population.

Methods: This study was conducted in an extended pedigree from the Netherlands. We used data from 802 individuals from a genetically isolated population. Carotid-femoral PWV was measured by the means of Complior® SP device. Natural logarithm transformation of PWV is used in the analysis. The variance component method implemented in SOLAR was used to estimate crude heritability (h^2) as well as age and sex-adjusted, and multivariable-adjusted heritability of PWV.

Results: Covariates used in the complete multivariable-adjusted model were gender, age, age^2, heart rate, systolic blood pressure, body mass index and smoking status. The crude and age- and sex-adjusted h^2 for PWV were 0.21 (SE=0.07, p<0.001) and 0.36 (SE=0.09, p<0.001), respectively. The multi-variable adjusted h^2 for PWV was 0.30 (SE 0.08, p<0.001). The proportion of variance due to all covariates was 0.55.

Conclusions: To our knowledge, this is the first report on heritability of PWV. Our findings suggest that a substantial part of variance in vessel wall stiffness is explained by genetic factors, opening the possibility to search for genes determining vascular structure using PWV as a useful measurement.

P1010. Relative contributions of the Caucasoid and Mongoloid components to the formation of Kazakhs as estimated from mitochondrial DNA polymorphism.

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The goal of the present research was to study the relative contributions of the Caucasoid and Mongoloid components to the formation of Kazakhs as estimated from mtDNA. DNA samples of 246 unrelated Kazakh men were taken in various regions of the Republic of Kazakhstan. The sequence of the first hypervariable segment of mtDNA was determined.

We found that 58 % of mtDNA lineages belonged to Asian-specific haplogroup (D, C, G, A, M, F). The supercluster D was found with high frequency (17,89%), compared to other populations of the Volga-Ural region: for Bashkirs D= 9,0%; Tatars D = 2,6%. East Asian hgs - C, G constituted about 16% in Kazakhs, compared with a frequency of C -11,8% and G - 4,5% in Bashkirs, C and G - 1,8% in Tatars. The Western-Eurasian specific haplogroups (H, T, J, K, U2, U5, HV) were observed in Kazakhs with a frequency of 42%. Haplogroup H was found with a frequency of 13% in Kazakhs, but this was less frequent than in Tatars - 30,7%.

In general, the results obtained agree with ethnic anthropological data indicating a stronger Mongoloid component (58%) and a lesser Caucasoid component (42%) in the Kazakh gene pool. The data obtained in this study allowed us to construct a phylogenetic tree for Kazakhs along the female lineage and to detect position of the studied population among ethnic groups in Europe and Asia.

P1011. Analysis of *CYP2A6*, *MAO* and *DBH* genotypes, smoking behaviour and cotinine levels in 1,528 adolescents of the UK School Heart and Health Study

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Smoking is the biggest risk factor for coronary disease and lifelong habit often initiates in adolescence. Inactivating mutations in *CYP2A6* which encodes the main enzyme which inactivates nicotine to cotinine, affect both cotinine levels and smoking behavior in adults. Diversity in genes relevant to dopaminergic and related pathways, which are important in addiction, has also been reported to be associated with smoking habits in adults. We have explored cotinine levels and initiation of smoking (self-reported questionnaire) in the UK-wide School Heart and Health Study (SHHS) in relation to CYP2A6, monoamine oxidase-A (MAOA) and dopamine beta-hydroxylase (DBH) genes.

We developed an integrated set of genotyping assays for five CYP2A6 alleles. We also examined promoter polymorphisms in the MAOA gene (T1460C and a minisatellite(VNTR) repeat) which have been observed to have epidemiological or transcriptional efficiency associations. We also examined DBH G1368A. A DNA bank representing 1,528 SHHS subjects was submitted to genotyping for all alleles, followed by genotype-phenotype analyses.

No cotinine nor smoking behavior associations were observed for DBH or MAOA genes. The integrated CYP2A6 assays enabled a more powerful study since each allele is individually rare. The total prevalence of nicotine slow metabolisers identified, aggregating CYP2A6 alleles *2, *4 and *5, was approximately 6%. Slow metabolisers showed 1.88-fold higher mean cotinine levels which may have a metabolic or behavioral basis. This appears paradoxical relative to studies in adults but may reflect non-saturating nicotine doses or different behavior during teenage experimenting phase.

P1012. APOE mutations: structural and evolutionary studies.

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In the course of population studies on apolipoprotein E (APOE) gene we found six different missense mutations. Two of them, Leu28->Pro and Thr42->Ala were found in subjects with sporadic late-onset Alzheimer's disease (AD); the other ones, Gly127->Asp, Glu204->Lys, Glu212->Lys, Val287-> Met, were found in apparently healthy subjects. Present work is an attempt to evaluate the possible impact of these mutations on the structure and function of the protein by modelling studies. The amino acid conservation across species was also analysed. The two mutations found in AD subjects,

Leu28->Pro and Th 42->Ala, located in the first helix (24-42) of the N-terminal domain, are in a highly conserved region among primates. Secondary structure predictions carried out on apoE sequences suggest that both mutations may significantly perturb the local structure. The three-dimensional structures of the N-terminal domain were also inspected to search for a possible influence of the two mutations. Again Leu28->Pro and Thr42->Ala are likely to affect the tertiary structure of the N-terminal domain. Since this domain has been suggested to bind the amyloid β-peptide (Aβ) and to be responsible for the stability of apoE/Aβ complexes, both substitutions could be involved in the apoE/Aβ interactions and therefore in AD susceptibility. As for Leu28->Pro, these findings are consistent with previous epidemiological data showing that subjects carrying it were at a significantly higher risk of developing AD, compared with all the other APOE genotypes, including those carrying e*4. Structural and evolutionary studies have been performed also for the other amino acid substitutions.

P1013. MtDNA analyses (HVRI and HVRII region polymorphisms) in skeletal samples of Thracian populations from Bronze and early Iron Age from Romania

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We inform on the study of the mtDNA (HVRI and HVRII region polymorphisms) from skeletal samples of Thracian populations dating from Bronze and early Iron Age, found in the SE of Romania and the implications of these data in understanding their genetic relationships with other old and modern European populations and their contribution to the foundation of the modern Romanian genetic pool. DNA has been extracted from 20 individuals (human fossil bones and teeth) using two methods, namely: the DNA extraction method with phenol/chloroform described by Hummel (2003, Springer Vrlg.) and the DNA extraction method with guanidine-tiocianat and silica particles describes by Hoss & Pääbo (1993, Nucl. Acids. Res., 21) adapted at the degradation state of biological material. Short fragments of the mtHVRI and mtHVRII were separately amplified by PCR, demonstrated by UV visualization by agarose gel electrophoresis, sequenced by Sanger method at MWG and screened for showing mutations in comparison with the modern mtDNA. So far, we have obtained mtDNA sequences from at least 11 Thracian individuals and identified 1 to 5 substitutions in comparison with the moderns Romanian population. The relative small number of genetic differences between the old Thracian and the modern Romanian populations suggests that the Thracian populations might have contributed of the genetic Romanian pool.

P1014. Detection of commonGJB2 (Connexin –26) 35delGmutation in theCroatian Population

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Congenital deafness affects approximately 1 in 1000 live births. More than 70% of hereditary hearing loss is nonsyndromic and among them about 75-80% are inherited in an autosomal recessive fashion. At least 20 genes that cause nonsyndromic recessive deafness have been localised. Mutations in the gene GJB2, encoding the gap-junction protein connexin 26, have been found in 50% of persons with severe autosomal recessive nonsyndromic sensorineural hearing loss. The GJB2 gene encodes the connexin 26 transmembrane protein. By oligomerization with five other connexin molecules it forms channels between cells which facilitates direct cytoplasmic-cytoplasmic exchange of electrolytes, second messengers and small molecules.

Several studies have shown that a single mutation in the GJB2 gene, frameshift 35delG, accounts for the majority of mutant alleles in different ethnic groups of Caucasian origin. The mutation is evident as a deletion of guanine within the stretch of six G's at +30 to +35 position of the cDNA. The deletion leads to premature chain termination.

The aim of our study was to determine the prevalence of the most common GJB2 mutation, 35delG, in patients with severe hearing impairment from Croatia. Patients were collected in collaboration with the Croatian referral centre for deafness. PCR amplification was carried out on genomic DNA and products were digested with BsiYI restriction enzyme. After digestion products were separated by electrophoresis on 3% agarose. Our preliminary results, obtained by analysis of 27 patients (54 alleles), indicated 35delG mutation in 17 (30%) alleles.

P1015. Phylogeography of Y chromosome lineages in North Eurasia

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Distribution of basic Y-chromosomal haplogroups in populations of Siberia, Central Asia, Far East and Eastern Europe was described. Y chromosome haplotyping was performed in 1614 individuals from 39 population samples belonging to 22 ethnic groups. Frequency of 24 monophyletic lineages was obtained. Most common haplogroups on the territory of North Eurasia are lineages R1a1 (25% of Y chromosomes in the total sample belong to this haplogroup), N3 (21%), C (10%), F (10%),and P (8%). Detailed analysis of molecular diversity within R1a1 lineage and phylogeographic analysis of its distribution were performed. Haplogroup R1a1 associated with dispersion of putative Indo-Aryan ancestors of modern Indo-Europeans has the highest frequency in Indo-Iranian populations including Tajiks and Uzbeks from Central Asia (20-25%), and in Eastern Slavs (25%). Among ethnic groups of Siberia R1a haplogroup is most frequent in populations of Altay-Sayan origin. Putative ancestral R1a1 haplotype in Altay-Sayan people is identical to one of the basic haplotypes in Slavs and Indo-Iranians and has the microsatellite repeat structure 16-25-10-11-12 at DYS19-390-391-392-393 loci. Approximate coalescent time for microsatellite R1a haplotypes to ancestral haplotype is 5500 (95% CI 3100-7000) years and corresponds to Neolithic ancient Caucasoid component in the origin of Altay-Sayan ethnic groups.

P1016. KORA: A Source of Population Controls for Genetic Studies

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Since 1985 population-based health surveys have been conducted in the region of Augsburg, Bavaria every five years. Data and biological samples are maintained by the KORA platform (Kooperative Gesundheitsforschung in der Region Augsburg) at the GSF, Neuherberg. The KORA cohort is a treasure of data and biological samples for studies on population genetics.

Recruitment of cases and their relatives for genetic family studies is difficult and costly, the power of linkage analyses is limited. Therefore, the case-control-design is becoming more and more prominent in genetic studies. However, the selection of controls is a crucial point in this kind of studies. During the first National Genome Research Network (NGFN), control populations from KORA have been used successfully in more than 30 case control studies. Due to the great interest, for NGFN-2 we have established an extended KORA control population of more than of 4000 controls in the age range of 25 to 84 years. DNA, basic phenotypic information and other biosamples (plasma, serum, blood) are available. The accumulating genotype information is made available to contributors of the database. Thus, fast access to a universal control population in anonymous form is combined with cost effective accumulation of relevant genotypic information.

We present here an overview about the scope of phenotypes and biological samples available for studies and a novel approach to selecting interactively via the KORA online portal a control group of choice for genetic analyses. In addition, the logistics and organisation of the KORA population genetic-control unit is discussed.

P1017. Evaluation of the GJB2 mutations in the Iranian population with autosomal recessive non-syndromic hearing loss

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Congenital hearing loss affects 1 in 1000 newborns and about 50% of all cases are estimated to be of genetic origin. About 70% of hereditary hearing loss is non-syndromic with autosomal recessive inheritance accounting for ~85% of the genetic load. 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) load in many populations of European descent. To assess the importance of GJB2 mutations in the Iranian population, we screened 556 probands with ARNSHL from different Iranian ethnic groups. Genetic testing began with an allele-specific polymerase chain reaction (ASPCR) assay to screen patients for the 35delG mutation (the most frequent deafness-causing *GJB2* variant in the people of Northern European ancestry). Patients heterozygous or negative for 35delG mutation were screened by DHPLC and sequencing for other GJB2 mutations. GJB2 related deafness was detected in 99 (17.8%) probands with an uneven distribution among different ethnic groups (from 10% in Baloochies to 41.7% in Mazandarani and Gilaki patients). Allele variants found are 35delG, delE120, 167delT, R184P, 310del14, V27I+E114G, R32H, 314del14, IVS1+1G>A, -3170G>A, R127H, W24X, R143W, M91I, V153I, V27I, 507insAACG, 329delA, I69I, 363delC. The 507insAACG is a novel mutation and the last three variants are novel unknown changes. Because the frequency of GJB2 mutations is much less than in the western populations, it is possible that other genes play a major role in ARNSHL in the Iranian population.

P1018. Age-independent extrinsic mortality has no influence on soma disposability. A remark on the evolution of aging.

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The disposable soma theory (Kirkwood 1977) states that intrinsic mortality, i.e., aging arises from a trade-off between reproduction and self-maintenance. A corollary of this theory predicts that species with high extrinsic mortality invest little effort in self-maintenance and age rapidly since preparation for life after extrinsic death would not be advantageous in evolution. However, as I show here, age-independent extrinsic mortality (h_{ex}) has no influence on self-maintenance and aging.

An age-structured population is described by the Lotka equation

$\int_0^\infty \exp(-r(\alpha)\, t)\, l(\alpha,t)\, m(\alpha,t)\, dt = 1$

with probability $l(\alpha,t)$ of survival to age t, rate $m(\alpha,t)$ of reproduction at this age, fitness $r(\alpha)$ (Hamilton 1966), and trade-off allocation parameter α. Introducing mortality $h(\alpha,t) = h_{ex} + h_{in}(\alpha,t)$ as the fractional decline in survivorship, $l(\alpha,t) = \exp(-H(\alpha,t))$ with $dH(\alpha,t)/dt = h(\alpha,t)$ and $H(\alpha,0) = 0$, results in

$\int_0^\infty m(\alpha,t)\, \exp(-(r(\alpha) + h_{ex})\, t - H_{in}(\alpha,t))\, dt = 1.$

Taking the derivative with respect to h_{ex} on both sides,

$\int_0^\infty -m(\alpha,t)\, (dr(\alpha)/dh_{ex} + 1)\, \exp(-(r(\alpha) + h_{ex})\, t - H_{in}(\alpha,t))\, dt = 0,$

implies that

$dr(\alpha)/dh_{ex} = -1$

because $m(\alpha,t)$ is non-negative. Hence, $r(\alpha) = -h_{ex} + F(\alpha)$. Thus, the difference, $r_A(\alpha_A) - r_B(\alpha_B)$, of two competing populations does not depend on h_{ex}, and neither does the evolutionary optimization of α, i.e., of the rate of aging. Although population biology arrived at this conclusion before (Charlesworth 1980), it was not translated sufficiently to the disposable soma theory (see Kirkwood and Rose 1991 or Cichon and Kozlowski 2000).

P1019. Laying the Cornerstone: Family Ascertainment in Genetic Epidemiological Studies

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Complex diseases such as Alzheimers, asthma or type-II diabetes have a higher prevalence than monogenic disorders in the general population. They cause a higher burden of illness and are thus of high socioeconomical relevance. However, studies on complex diseases tend to be rather complex themselves. They are frequently multicentred, joining up different medical institutions and clinical researchers with geneticists and epidemiologists. It is self evident that any statistical analysis can only be as valid as its data and the foundation it rests on, that is sound epidemiological field work. In addition, carefully phenotyped and genotyped study populations are an important asset for future research of functional genomics.

The logistic complexity for various genetic studies in the German National Genome Research Network is coordinated by special competence centres for Genetic Epidemiological Methodology (GEM). GEM Munich works in close collaboration with clinical partners and manages the complete logistics of family recruitment, of information ascertainment from family members and its integration with genetic data from high-throughput genotyping facilities.

In this work we present our approach to the successful organisation of field work logistics and quality control and discuss the various choices and options available. To distribute information between stakeholders more efficiently, we have developed an online system for recruiting fieldwork with remote data entry capabilities, forming a “Virtual Study Centre”. Our integrated approach ensures large number family-recruitment for successful research on complex diseases.

P1020. Ancient DNA Alu-insertion polymorphism in Siberian pra-Selkup culture people

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Pra-Selkup archeological culture dated back to XII-XVII centuries was dispersed on the territory of middle Ob River (Westen Siberia) but the genetic relationships of pra-Selkup people with ancient and modern populations of native Siberians are not known. We performed the ancient DNA analysis in 20 pra-Selkup men from burial grounds from middle Ob River region. Total DNA was extracted from skull bones of 20 men by a phenol - chloroformic method with the subsequent cleaning on Centricon 100. The DNA was analysed using a set of primers for 4 autosomal loci of Alu insertion: ACE, APOA1, FXIIIB, PV92. Some genotypes have not been established because of weak amplification of alleles with the length >400 bp (table). Thus, minimal (MIN) frequency of insertion (p(I)) at the locus ACE was 0.175, MAX p(I)=0.30. APOA1 genotypes were: II=19; 00=1, in accordance with high insertion distribution in the populations worldwide (0.70 – 0.99). For FXIIIB and PV92 loci frequency of Alu were: MIN p(I)=0.175; MAX p(I)=0.625 and MIN p(I)=0.35; MAX p(I)=0.45), accordingly. The Asian populations and aboriginal Siberian populations were characterized by relatively high frequencies of the Alu-repeat insertion at these two loci (0.53 – 0.70). Our data on the reduced insertion frequency at the FXIIIB and PV92 loci in pra-Selkup people can be explained by the contribution of Caucasoid components in formation of Selkups or can be caused by the stochastic reasons. Further investigation of mtDNA and Y-chromosome lineage in pra-Selkup could put more light on pra-Selkup genetic ancestry.

Alu genotypes at the ACE, FXIIIB, PV92 loci (I - insertion, D - absence Alu-repeat, 0 - NS)

Genotype/ Loci	ID	DD	D0
ACE	7	8	5
FXIIIB	7	-	8
PV92	14	2	4

P1021. Haplotype structure of the VDR genomic region in three human populations

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Recent findings show that human genome is organized as discrete regions that are transmitted as blocks. The haplotype structure of these blocks could be defined analyzing the linkage-disequilibrium (LD) of SNPs distributed along it. The characterization of the haplotype structure of genes associated with complex human diseases is of great interest. Variations in the Vitamin D Receptor (*VDR*) gene have been associated with several diseases. Some of these findings have been replicated in different human populations with discordant results. In addition, there is interest to clarify if *VDR* variants are associated with the condition because either they are biologically causal, or they are statistically correlated with a causal variant. In the present work we have studied a region of 220 Kb surrounding the *VDR* gene included in the NT_029419 contig at 12q12-q13. Haplotype structure of this region has been defined by analyzing LD patterns of 24 SNPs distributed along it. Genotypes has been determined for three human populations: a Sub-Saharan sample of 56 individuals, a south European sample of 200 individuals and a north European sample of 97 individuals. According LD patterns three regions with D' > 0.75 were observed in the two European samples that allow us to define three blocks. In contrast, the Sub-Saharan sample shows low levels of LD that precludes the clear identification of blocks in this sample. The block pattern defined in the two European samples indicates that both *VDR* and the two neighbouring genes *HDAC7* and *COL2A1* are located in different blocks.

P1022. Analysis of ACE, AT1R, eNOS, MTHFR, MTRR and ApoE genes polymorphisms in the population of North-West of Russia

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The search for genetic factors responsible for inherited predisposition to some common multifactorial diseases has become a major goal of molecular medicine. Estimation of population-specific frequencies of relevant polymorphisms in normal control population remains the major prerequisite of these studies. The polymorphisms of seven genes (I/D-ACE, A1166C-AT1R, 4a/4b-eNOS, 677C/T-MTHFR, A66G-MTRR, APOE) associated with some multifactorial diseases (cardiovascular diseases, diabetes) were studied by PCR-RFLP analysis in North-West population of Russia. The group consisted of unrelated individuals of middle age (25-45) (59 men and 58 women). Distribution of relevant polymorphisms frequencies for MTHFR, MTRR, APOE, eNOS and AT1R genes was similar in our sample compared to these ones in some European populations (Spain and Germany). Low frequency of D/D genotype and high frequency of I/I genotype were found in Russian compared to Spain population (D/D - 29,6% and 41%, I/I - 26,1% and 15%, respectively). No significant statistical differences in distribution of ACE polymorphisms was found between Russian and Scotland populations (D/D-31%, I/I-20%). Thus population specific differences for the polymorphisms of some genes and its obvious similarity for the other ones were disclosed.

P1023. MMP3 gene (stromelysin-1) polymorphism (5A/6A) and stroke in Serbian population

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Background: MMP-3 is part of the extracellular matrix metalloproteinases family. It is a key player in extracellular matrix degradation and remodeling. Homozygote for MMP-3 gene 6A allele have decreased level of stromelysin-1 which can lead to acumulatation of extracellular matrix in the blood vessel wall and progression of atherosclerosis. Homozygote for MMP-3 5A allele have increased level of stromelysin-1 and its greater proteolytic activity leads to plaque instability and possible rupture.

Aim: The aim of this study was to determine whether the MMP-3 promoter polymorphism 5A/6A represent risk factor for stroke in Serbian population.

Methods: We studied 59 patients with stroke and 96 healthy controls. The presence of the polymorphic genotypes were determined by polymerase chain reaction and restriction enzyme digestion with *PdmI* restriction endonuclease.

Results: Frequencies for 5A/5A, 5A/6A, 6A/6A genotypes was 15.25%, 49.15% and 35.6% in patients and 5.2%, 62.5% and 32.3% in controls. Significant difference in MMP-3 genotypes frequency distribution was found between healthy controls and patients with stroke ($p<0.05$). Allele frequencies were not significantly different between patients and controls.

Conclusion: Our results suggest possible role of MMP-3 5A/6A gene polymorphism in occurrence of stroke. We found higher prevalence of 5A/5A genotype in the group of patients with stroke compared to healthy controls which confirms his role in the plaque instability. This observation needs further confirmation through larger sample studies in patients with stroke.

P1024. Common ancestor or not. Origin investigation

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A patient came to us with the target to track down his family that had drifted apart during the course of history. His request was that we make an attempt to identify the family members of the same name he thought to have discovered. His geneologic researches led him to believe that a presumably common ancestor lived in the XVI. century and was a known historical figure, which could be tracked down to one of the branches of the family tree. For verification, as a starting point, the DNA study of the Y chromosomes was carried out in case of the male family members bearing the same name, since this should give identical results, presuming legitimate origin. Chromosome polymorphism analysis was also parallel performed for both male and female members. The individuals who had not known each other earlier thought to have discovered similarities on each other, therefore antropologic studies were also conducted upon their request.

Based on the obtained results, it is assumable that the studied individuals are related to each other, although certain mutation deviations from the 12 studied markers were found during the DNA studies. The mutation frequency was assumed to be 1/500 generations on average for each marker. The generation time of the nearest common ancestor of two individuals can only be estimated with a rather big error limit, therefore when researching family trees care should be taken in estimating the time of genetic separation of two male branches.

P1025. Multiplex PCR for the Assignement of Some Major Branches of the Y Chromosome Tree

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We have set-up a multiplex PCR reaction for the fast genotyping of human male DNA for six major branches (D, E, G2, I, J and J2) of the Y chromosome tree. The reaction was designed specifically for lineages which are prevalent in Southern European populations. Six markers (YAP, P15, M170, 12f2a, M172 and P27) were amplified in the reaction. Each sample can be assigned to one of the discussed branches, either directly (presence/absence of the specific PCR product), or after a further dot-blot hybridization step with specific probes. The technique provides a reliable effort-effective and time-effective method for Y chromosome genotyping for the six above markers.

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P1026. DHPLC approach to determine RHD variants molecular epidemiology in Brittany (France)

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Since the first description of molecular genetic bases of Rhesus D blood group phenotypes, more than 90 molecular variants have been described. According to the location of the amino-acid change in the protein (extracellular loops vs. transmembranous or intracellular parts), a genotype-phenotype correlation is possible.
The aim of this study was to evaluate the molecular epidemiology of RHD variants in Brittany (western France) using DHPLC, a sensitive, rapid and automatable technique.
Along year 2003, during blood donor typing at the EFS-Bretagne, 73 samples presenting a discordant Rhesus D serology were analyzed at molecular level. Hybrid alleles were typed with an exon specific PCR whereas single nucleotide variations were screened by DHPLC and identified by sequencing.
Among 11 variants encountered, we were able to identify 2 new variants (IVS3+5G>A and p.A226D) despite the low number of samples analyzed. Unexpectidly, half of the partial D were D catV type VII (16%) which was known as a very rare allele. Among 7 Weak D alleles, type 1 (37%) and 2 (22%) were predominant.
DHPLC analysis for the RHD gene would allow to extend the population studied in order to confirm the original molecular epidemiology we found in Brittany. Furthermore, this study highlights the interest for transfusion couselling of molecular typing for samples with ambiguous serological results as we identify Weak D (type 4.2 and 15) and new partial D (A226D) which are prone to alloimmunisation.

P1027. The load of monogenic diseases in Siberian populations

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The data of population and medical genetic investigation of monogenic diseases (MD) in native and Slavonic Siberia populations are presented. In the native populations, the load of autosomal dominant (AD), autosomal recessive (AR), and X-linked pathology was accordingly $0.58x10^{-3}$, $0.92x10^{-3}$ and $0.49x10^{-3}$ in Tuvinians; $0.73x10^{-3}$, $1.22x10^{-3}$ and $0.11x10^{-3}$ in Altaic populations; $1.7x10^{-3}$, $0.7x10^{-3}$ and $0.4x10^{-3}$ in Yakuts. The load of AD, AR and X-linked pathology in Slavonic population of Tomsk region was $0.84x10^{-3}$, $0.82x10^{-3}$ and $0.39x10^{-3}$ respectively. The spectrum of MD in separate Siberian ethnic groups was characterized by specificity of some forms of hereditary diseases. High frequencies of definite mutations are revealed in some native Siberian populations. In particular, spiniocerebellar ataxia-1 (SCA1 - MIM 164400), dystrophia myotonica (DM - MIM 160900) and methemoglobinemia type 1 (NADH-cytochrome b5 reductase deficiency - MIM 250800) are widely spread within the Yakutian population. In Tuvinian and Altaic populations we observed a high frequency of microtia with meatal atresia and conductive deafness (MIM 251800). The present study give us the opportunity to create a prophylactic register of MD and come to the prospective genetic counselling in high-risk families with monogenic disorders.

P1028. Study of a consanguineous population from Banat county

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Gene frequencies in small isolate populations are different from those of the general population, due to a founder effect. Founder effect occurs when the population grew from a few founding individuals. When the founding population is small, intermarriage must result. The mutations carried by the founders are in higher frequency than they would be in the general population. The aim of the present study was to demonstrate the correlation between the level of consanguinity and the pathological profil in an isolated population from Banat county, Romania. The study was performed on 250 from the 400 individuals of this isolate. Historical data reveal that the isolate was formed between 1823-1825. The genetic pool was preserved over a century and a half. Geografic (the teritory is surrounded by Danube and mountains), ethnic(Czech population) and religious (catholics) barriers caused a high inbreeding and consanguinity coefficient in this population. The pathological profil of the community is characterized by a high incidence of neuropsychic disorders (12%, half of them being mentally retarded). Morbidity and mortality in children are four times higher than in the general population. Plurimalformative syndroms were seen in 14.8% of the individuals and inborn errors of metabolism in 4.6%. Matrimonial traditions are maintained until today, but the community becames smaller and smaller due to the intense migration in the last years.

P1029. Mitochondrial haplogroup and association to other disease in Iran.

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We studied 70 MS patients and 149 normal healthy controls for Haplogroup J and K with PCR RFLP method. Our result showed that 14 out of 70 (20%) in MS group and 14/149 (9.4%) in control group were positive for J ($P < 0.05$); and 9/70 (13%) in MS group and 5 out of 70 (7%) in control group were positive for Haplogroup K ($P=0.4$). This study showed that maybe some mtDNA Haplogroups are a risk factor for MS diseases but genetic susceptibility factors for these disorders vary between different populations. Association between Haplogroup J and Optic Neuritis was significant ($P< 0.005$).
Using haplogroup specific restriction site analysis (PCR-RFLP), we compared the polymorphisms in Iranian LHON patients and a normal control group (149 controls for haplogroup J. and 246 controls for haplogroups M, BM and N, from different regions of Iran). In contrast to some other reports, we did not observe any relation between LHON and the haplogroups J, M and N. However the results showed a slight relatedness between haplogroup BM and LHON. Interestingly the Asian marker M was not so frequent in normal controls, while the frequency of European marker J in normal controls was similar. As a result, this study gives evidence for similarity between Iranian population ethnic groups and people from northwest Asia and southeast Europe.

P1030. Nucleotide sequence variation and haplotype structure of the ICAM1 and TNFa genes in Indian populations reveal different signatures of natural selection

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We have examined the patterns of DNA sequence variation in and around the genes coding for Intercellular Adhesin Molecule 1 (ICAM1) and Tumor Necrosis Factor a (TNFa), which play functional and correlated roles in inflammatory processes and immune cell responses, in 12 diverse ethnic groups of India, with a view to quantifying the nature and extent of the variation and to investigate into the causes of maintenance of the observed patterns of variation. At the ICAM1 and TNFa loci, respectively, the total numbers of SNPs that were detected were 28 and 12. Many of these SNPs are not shared across populations. Many remain unreported in dbSNP or TSC databases, including two fairly common non-synonymous ones at nucleotide positions 13487 and 13542 in the ICAM1 gene. Wide between-population variation in the frequencies of shared SNPs and coefficients of linkage disequilibrium have been observed. At the ICAM1 locus, the observed excess of intermediate-frequency alleles and the structure of phylogenetic network among haplotypes provide strong evidence of balancing selection. On the other hand, at the TNFa locus, the observed patterns of sequence variation are similar to those expected under population expansion (excess of rare alleles, unimodal mismatch distribution, starlike phylogeny of haplotypes). These inferences regarding the distinct causes of maintenance of variation in these two genes are consistent with their known modes of action.

P1031. Investigation of Mitochondrial Haplo group J, M, N, BM in Iranian population.

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Mitochondrial DNA (mt. DNA) haplogroups M, BM, and N, were examined in 246 individuals from the North (41), East (20), West (48), Centre (22), and South (17) of Iran.
Interestingly the main Asian haplogroup M was not seen at high frequency (2.4%) in the Iranian population; its frequency was highest in west and central Iran (3.8% and 4.5% respectively) and lower in the south and east of Iran (0.0%). The frequency of the European marker J was 9.6% in normal controls. As a result, this study gives evidence for similarity between the ethnic groups of the Iranian population and people from northwest Asia and southeast Europe. Our data suggest that Iranian tribes probably played a significant role in the formation of these ethnic groups. Haplogroup J may be older than 66,000-10,000 years, and probably developed in Iran, and then expanded to different regions of Europe and northwest Asia. On the other hand it seems that the super-haplogroup M developed after the inhabitants of Iran moved to the east of Asia, or this group migrated from south of Iran/north of Arabian gulf to Pakistan and then to Asia.

P1032. Prevalence of Factor V, Factor II, and MTHFR gene mutations in Lebanese patients: report from a tertiary care center

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Physicians are increasingly aware of the significance of testing for Factor V, Prothrombin, and MTHFR mutations in several medical and surgical conditions. The frequency of Factor V Leiden in Lebanon is the highest recorded in the world so far. We therefore screened for Factor V Leiden and mutations in Factor II and MTHFR in 207 patients referred to a tertiary care center in our country over a one-year period. Of these, 47.83% were referred from Internal Medicine, 21.26% from Surgery, 12.08% from Pediatrics, 11.11% from Obstetrics and Gynecology, while in 7.72% of cases the reason for referral was not mentioned. Testing for all three mutations was done using the Reverse Hybridization StripAssay test (ViennaLab). In our patient population, homozygosity for Factor V, Factor II, and MTHFR was found to be 4.83%, 0%, and 9.66% respectively. Heterozygosity was found to be 28.5%, 9.18%, and 41.06% respectively, while its frequency in healthy individuals has been reported as 14%, 3%, and 38%. The highest prevalence of double heterozygosity was for Factor V Leiden/MTHFR mutations (10.15%), while other double heterozygous combinations were less frequent (4.35% for Factor V Leiden/Factor II mutations and 2.9% for Factor II/MTHFR). Only one double homozygous case for Factor V/MTHFR was found. The higher frequency of mutations in these genes among patient populations seems to warrant routine screening for proper intervention and genetic counseling.

P1033. SNP haplotypes in the LPA gene show association with Lp(a) levels and are in linkage disequilibrium with K IV numbers

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LPA the gene coding for apolipoprotein(a) is the major determinant of lipoprotein(a) plasma levels which are associated with risk for CHD. It is not completely understood how variation in LPA relates to Lp(a) concentrations. One type of variation related to Lp(a) levels is the number of K IV-2 repeats in LPA but sequence variation may also contribute. Here we have studied the effect of two SNPs (C93T and G121A) in the LPA gene. Because of the short distance between these SNPs it was possible to design an assay which allows the simultaneous detection of both SNPs and thus of haplotypes. Using this assay genomic DNA was analyzed in 149 Asian Indian and 52 Gabonese subjects of which Lp(a) levels and K IV-2 repeat numbers were available. Mean Lp(a) levels were significantly different in homozygotes for the SNP haplotypes but the association was opposite in the two populations. 1-1 genotypes were associated with high Lp(a) in the Indian population but with low Lp(a) in the African population. In addition linkage disequilibrium between the haplotypes and the number of K IV-2 repeats was observed. In both populations “1” haplotypes were present on LPA alleles with low number of K IV repeats and “2” haplotypes on large apo(a) alleles. Further analysis revealed that in the Indian population the association between SNP haplotypes and Lp(a) levels was completely due to the linkage disequilibrium between SNP haplotypes and K IV number but a different mechanism must explain the association in the African population

P1034. Genetic characterization of Albanian historical ethnic minority of Calabria (Southern Italy)

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Three ethnic minorities are present in Calabria: Albanian, Greek, and Occitan. The Albanian ethnic minority is the more populous settled in Calabria between the XV and XVII centuries A.D., and these people are now located in the provinces of Cosenza and Catanzaro. In the present work the Albanian population structure is analysed based upon the allelic frequencies of six classic genetic markers: ACP, GC, PGM1, AK, ADA, and 6PGD. The results show a significant heterogeneity between the Albanian population in Calabria and the population in Molise. Therefore, the cultural and mating isolation of the Albanian ethnic minority of Calabria is related to a great genetic peculiarity. Moreover, the frequencies of some alleles, particularly that of the PGM*1W31 variant, and the analysis of the R-matrix show still today the actual peculiar genetic structure of the Albanians of Calabria, although the genic flow which is evidentiated by the decrease of the endogamy, and by the degree of mixing

P1035. Intrauterine exposure to folic acid antagonists and the risk on folic acid sensitive congenital anomalies

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Background: The protective effect of folic acid (FA) on neural tube defects (NTDs) and other anomalies is well known. If FA prevents congenital anomalies, it is reasonable to assume intrauterine exposure to FA antagonists increases the risk on these FA-sensitive defects.
Objectives: Using data from the EUROCAT Northern Netherlands registry, we have investigated the possible harmful effect of FA-antagonists on FA-sensitive defects: congenital heart defects, NTDs, clefts, urinary anomalies, limb reduction defects, anal atresia and omphalocele.
Methods: Case-control analyses were performed using children and foetuses born from 1997 through 2002. FA-antagonists are divided into two groups: dihydrofolate reductase inhibitors (DRI) and antiepileptics. Not exposed was defined as no use of any FA-antagonist during the first 10 weeks after lmp. Cases were defined as having one of the FA-sensitive defects. Controls were all other defects including all chromosomal or monogenic disorders (n=1729).
Results: For all FA-sensitive defects the study showed no effect after exposure to a FA-antagonist (OR=1.21, 95% CI: 0.56-2.62). We found no effect of exposure to a DRI on FA-sensitive defects (OR 0.44, 95% CI: 0.13-1.57) but we did find a significant effect after exposure to an antiepileptic (OR=3.52, 95% CI:1.06-11.72). Among the subgroups we found significant effects after exposure to antiepileptics for congenital heart defects and NTDs.
Conclusions: This study supports the findings of various other studies on the teratogenicity of antiepileptics. Birth defect registries will continue to monitor the future effect of FA-antagonists on birth defects in order to determine their possible harmful effects on congenital anomalies.

P1036. Single nucleotide polymorphisms in genes relating to homocysteine metabolism: How applicable are public SNP databases to a typical Caucasian population?

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To facilitate the association studies in complex diseases characterized by hyperhomocysteinemia we collected structural and frequency data on SNPs in 24 genes relating to homocysteine metabolism. Firstly, we scanned ~1.2 Mbp of sequence in the NCBI SNP database build 110, and we detected 1353 putative SNPs with an average *in-silico* genic density of 1:683; 85 entries were contained in coding regions (cSNPs). Using a subset of 42 cSNPs we assessed the applicability of the NCBI SNP database to the Czech population- a typical representative of Central European Caucasians- by determining frequency of the putative cSNPs experimentally by PCR-RFLP or ARMS-PCR in at least 100 control Czech chromosomes. As only 25 of the 42 analyzed cSNPs met the criterion of ≥1% frequency, the positive predictive value of the NCBI dataset for our population reached 60%, which is similar to other studies. The correlation of SNP frequency between Czechs and other Caucasians- obtained from NCBI and/or literature- was stronger (r^2=0.90 for 20 cSNPs) than between Czechs and general NCBI database entries (r^2=0.73 for 27 cSNPs). Moreover, frequencies of all 20 putative cSNPs, for which data in Caucasians were available, were congruently below or above the 1% frequency criterion both in Czechs and in other Caucasians. In summary, our study shows that the NCBI SNP database is a valuable tool for selecting markers for genetic studies in hyperhomocysteinemia in European populations although experimental validation of SNPs should be performed, especially if the cSNP entry lacks any frequency data in Caucasians.

P1037. Complete analysis of the dihydropyrimidine dehydrogenase gene in a control cohort of Caucasian individuals

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Germline mutations in the dihydropyrimidine dehydrogenase gene (DPYD) have been associated with inactivation of the enzyme dihydropyrimidine dehydrogenase (DPD), which is involved in the catabolism of the anticancer drug 5-fluorouracil (5-FU). We performed a population study to evaluate the frequency of sequence variations in the DPYD gene in a Caucasian population. Therefore, a control cohort of n=157 individuals was analyzed by denaturing HPLC. Some of the sequence alterations detected in the normal population displayed high allelic frequency: the alterations 85T>C (C29R) and 1627A>G (I543V) were observed in heterozygous as well as homozygous state with frequencies of 19.4% and 13.7%, respectively. Alleles containing the sequence deviations 496A>G (M166V), 1896T>C (F632) or 2194G>A (V732I) were also common. The mutation 1601G>A (S534N) which is so far controversially discussed as a common polymorphism or as a variant implicated in 5-FU intolerance, appeared in an allelic frequency of 1.6%. Some rare mutations were only found in heterozygous state in one or two individuals. Among these variants, a frameshift mutation (295-298delTCAT) and three novel mutations, 1218G>A (M406I), 1236G>A, (E412), 3067C>T (P1023S), were detected. Finally, the variant 2846A>T (D949V) previously reported in a patient with DPD-deficiency, was confirmed in two subjects of our study. A statistically significant deviation from the median DPD activity of the control cohort was evident for carriers of the mutations 1601G>A, 496A>G, and 2846A>T. This work presents a complete analysis of the DPYD gene in a large cohort of Caucasians and may contribute to further elucidation of the pharmacogenetic disorder of DPD deficiency.

P1038. Haplotypes of the NF1 gene indicate that the European population is a mixture of two genetically different ancient subpopulations

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The genetic structure of the common European population is still under debate, though it has been intensely analysed, mostly by using mitochondrial and Y-chromosomal markers. Here we report the results of an analysis of the haplotype structure of the NF1 gene. This autosomal locus is optimal for population genetic studies because it is a highly conserved, 350 kb long, structurally and functionally defined genomic unit with an extremely low recombination rate. Two groups of haplotypes (haplogroups) were detected by SNP analysis and resequencing of intronic parts of the NF1 gene in the German population, which can be regarded as representative for the common European population. The sequences of the two haplogroups are clearly separated from each other, with one divergent site per 1200 nucleotides. Haplogroup 1 sequences, which comprise 67 % of the chromosomes analysed, show very little intragroup variability and a starlike phylogenetic tree, characteristic of a population, which went through a severe bottleneck followed by a rapid expansion. Haplogroup 2 sequences, 33 % of analysed chromosomes, show deeper splits into a number of subgroups, a typical pattern for a population with a stable size for a longer period of time. These variability patterns clearly indicate that the recent European population is a mixture of two ancient subpopulations. One third of the European gene pool may be derived from the first settlements of modern humans in Europe, whereas the larger part of the gene pool may represent a later immigration and expansion of a population of neolithic farmers.

P1039. Genetic predisposition to coronary artery disease in Turkish population -preliminary findings

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Background : The origin of artery disease lies in interaction between genetic predisposition and environmental factors. The environmental factors that may cause coronary artery disease have been throughly investigated but the role of genetic markers is still poorly understood. This study investigates the relation between coronary artery disease (CAD) and Factor V His1299Arg (FV A4070G), Prothrombin 20210A, MTHFR C677T and A1298C gene mutations in Turkish population.
Method : 99 CAD patients (70 men & 29 women) and 30 control subject (17 men & 13 women) were studied. FV A4070G, prothrombin 20210A. MTHFR C677T and A1298C mutations were screened using PCR-RFLP methods.
Results : The incidence of MTHFR 677TT genotype was found %12,1 in CAD patients and most common in women with CAD (6/29; 20,7%). MTHFR 677CT frequency was determined as 42,4% in patient group. In control group MHTFR 677CT and TT frequencies were found 36,6% and 3,3% respectively. Incidence of MTHFR 1298CC and AC genotype were detected as 50,5% ; 5,5% in patient group and 52,3 %; 13,3% in control group respectively. Prothrombin 20210A and FV A4070G polymorphism detection results under evaluation.
Conclusion : MTHFR C677T polymorphism is relatively common in women with CAD. But the number of subjects is not sufficient yet for reasonable statistical analysis. However more studies are needed in order to clarify the association between prothrombotic genetic factors and arterial thrombosis.

P1040. mtDNA variability in populations of the Caucasus

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With more than 50 populations and dozens of distinct languages, the Caucasus is one of the most complex linguistic and ethnic mosaics in the world. Mitochondrial DNA and NRY variability was studied in 17 Caucasus populations speaking Caucasus, Turkic, Indo-European and Oirat Mongol languages. As a background, similar data about large variety of populations living north and

south of the area was analyzed. Total sample includes more than 1700 individuals. Mountain inhabitants of Dagestan reveal a great distinction possibly appears due to low level of interethnic marriages basing on mtDNA analysis. Analysis of NRY SNPs reveals extremely low diversity as far as SNP-defined haplogroups are concerned. This result depends on the depth of an analysis - maybe it is just a problem of scarcity of appropriate markers for this region. Pattern and frequency of observed mtDNA haplogroups in mountain populations is similar to Western Eurasian populations. As regards the steppe inhabitants mainly Karanogays, this population demonstrate up to 40% of Eastern Eurasian mtDNA lineages in contrast to their neighbors, even Kuban Nogays (up to 10%). This fact can be construed as preservation in this population of genetic features of ancient Nogai nomads as part Mongol Empire. 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. Also possible migration routs, peopling of steppe and mountain parts of the Caucasus and causes of high linguistic diversity presence in this region is analyzed in this study.

P1041. Analysis of Alu insertions in Altaic and Finno-Ugric speaking populations

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We have analyzed 9 Alu loci (Ya5NBC5, Ya5NBC27, Ya5NBC148, Ya5NBC182, Ya5NBC361, ACE, ApoA1, PV92, TPA25) in populations of Volga-Ural region (Trans-Ural Bashkirs, Tatars-mishars, Mordvinians-moksha, Mari, Udmurts, Komi-permyaks), in populations of Central Asia (Uzbeks, Kazakhs, Uighurs), and populations of the North Caucasus (Kuban Nogays, Karanogays, Karachays, Kumyks). All Alu insertion loci were polymorphic in all analyzed populations, allele frequencies varied from 0,110 in Mari on Ya5NBC5 locus to 0,914 in Tatars on ApoA1. The distribution of allele frequencies on 9 loci showed presence of substantial genetic diversity in studied populations. The average heterozygosity for 9 Alu insertions ranged from 0,342 in Mordvinians up to 0,450 in Kazakhs and Uighurs. The genetic differentiation (Gst value) among populations of Volga-Ural region was 0,040, and was much higher than in Central Asia and the North Caucasus (Gst=0,013). The studied populations appeared to have high differentiation both on linguistic and geographical features. Populations of Central Asia showed relative homogeneity of Alu insertions frequency distribution. Tatars differs from all analyzed populations except Kuban Nogays, what can support the fact of admixture when the latter were nomads. In a whole eastern Eurasian populations are rather similar to each other; the same concerns to Central Asian groups. Principal component analysis showed exact clustering into one group of Finno-Ugric speakers of Volga-Ural region. At the same time some of Altaic speakers inhabiting different region (i.e. Tatars, Kumyks, and Karachays) formed different cluster which is support the hypothesis of there common origin.

P1042. Autosomal DNA diversity of Eastern Europeans revealed by five HVR loci polymorphisms

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A study of DNA diversity is very important to understand the nature of human populations relationships. Eastern European ethnic groups relationships were investigated using the polymorphism of five markers with tandem repeat structure. These autosomal DNA markers are extremely polymorphic and are widely used in medical, forensic and population genetic studies. Eastern European region inhabited by a number of ethnic groups belongs to two main anthropological types and their admixtures. The study encompasses 1980 unrelated natives from 10 ethnic groups, namely Adygeys, Russians, Ukrainians, Belorussians, Kalmyks, Yakuts, Komis, Mordvinians, Bashkirs, and Udmurts.
The analysis of DNA minisatellite polymorphisms (3'ApoB and D1S80 loci) was carried out using the PCR and subsequent electrophoresis followed by silver staining. The triplet microsatellites (DM, DRPLA, SCA1 loci) variability was investigated using ^{33}P-labelling of PCR primers. Allele distributions of the loci studied were in agreement with Hardy-Weinberg expectations in all populations. All of the loci studied shown high polymorphism level and heterozygosity above 67%. The data treatment includes multidimensional scaling of Nei's genetic distances between ethnic groups. An UPGMA and NJ trees were constructed also. Observed cluster patterns reveal maximum distance between Caucasoids and Mongoloids of East Europe, while Uralic populations are intermediate and most closely related to Caucasoids. The data obtained are in agreement with ethno-historic and linguistic studies of the East European region and may contribute in evolutionary research of genetic relations between European and Asian populations.

P1043. The nine pericentric inversions that distinguish the karyotypes of human and chimpanzee predate the separation of Pan paniscus (bonobo) and Pan troglodytes lineages.

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Although human and chimpanzee chromosomes are very similar, some notable variations can be observed. This is the tandem fusion of two submetacentric chromosomes that occurred specifically in the human lineage giving rise to chromosome 2. Furthermore, nine pericentric inversions that constitute a difference between human and chimpanzee karyotypes are seen when comparing the G-banded chromosomes. These rearrangements attract much interest, since they must have occurred during the last 5 million years and possibly have influenced parapatric speciation of early hominins and chimpanzees, both cohabited in East Africa. Breakpoint analysis of the pericentric inversions between human and chimpanzee is a basic prerequisite to disclose their role during evolution. During our ongoing Homo sapiens/ Pan troglodytes comparisons, we have determined breakpoint spanning BACs to identify seven of these rearrangements (chromosomes 4, 5, 9, 12, 16, 17, 18) and a diagnostic BAC in case of chromosome 1. In order to investigate whether the bonobo (Pan paniscus) has the same pericentric inversions as the common chimpanzee (Pan troglodytes) we used fluorescence in situ hybridization. We proved that these BACs detect the same inversion breakpoints in Pan paniscus and in Pan troglodytes chromosomes. The obtained results lead to the conclusion that the fixation of multiple pericentric inversions predates the separation of both lineages and thus occurred in the relative short interval from 5 to 1,8 Mya.

P1044. Identification of novel polymorphisms and analysis of haplotypes in the methionine synthase reductase gene.

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Methionine synthase reductase (MTRR) is an enzyme required for the reductive activation of methionine synthase in the remethylation pathway of homocysteine metabolism. MTRR deficiency causes a rare autosomal recessive disorder, the cblE type of homocystinuria. Several pathogenic mutations and two common polymorphisms were previously found in the gene encoding the human MTRR. The aim of this study was to identify novel polymorphisms in the MTRR gene, to determine their frequencies in the Central European and sub-Saharan populations, and to analyse the MTRR haplotypes.
By sequencing of RT-PCR products we found 16 polymorphisms in exonic regions- 2 known and 14 novel. Six polymorphisms were missense mutations (M22I, S175L, S257T, K350R, R415C, H595Y), eight variants were synonymous changes and two variants were located in the 3´-UTR of the MTRR cDNA. Using PCR-RFLP analysis of 400 control alleles from two distant populations we determined frequencies of the variant alleles in the range from 0.02 to 0.44. Results from these studies were utilised for haplotype analysis using

statistical program Hapmax, which predicted 12 most probable haplotypes. Eight haplotypes were common for both populations and two were private for each population. Using maximum parsimony method it was also possible to deduce an evolutionary tree of MTRR alleles with a putative ancestral haplotype.
Although our data show that the MTRR gene is highly polymorphic, there is a high degree of linkage disequilibrium among polymorphisms as relatively few haplotypes were found. The clinical significance of haplotypes and polymorphisms in the MTRR gene, however, remains to be determined.

P1045. Haplotype reconstruction methods: comparison of three algorithms

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Owing to the rapid technological advance over the past years, a huge amount of genetic data is available for analysis. Therefore, effective methods must be developed that can both handle a large quantity of data and protect from the multiple testing problem. Haplotypes combine the information of several polymorphisms and thus meet the above criteria. We compare three methods to infer haplotypes, namely the EM algorithm implemented in SAS/Genetics, the PHASE algorithm, and the partition ligation EM algorithm. Our analysis bases on seven polymorphisms in the *Calpain-10* gene. The EM algorithm and the partition ligation EM algorithm come to quite similar results. Expected frequencies of the most prevalent haplotypes (above 10%) differ in the order of up to 0.5 percentage points. In contrast to these procedures, the PHASE algorithm is based on a Bayesian technique called Gibbs sampling. The results of PHASE deviate in some degree from the other two programs. The expected frequencies of the most common haplotypes calculated with PHASE show a deviation of up to 2.5 percentage points from the results of the procedures based on the EM algorithm.
Simulations are used as a further means to validate the haplotype reconstruction methods. The EM algorithm yields better results than PHASE in terms of accuracy and robustness.In a further step, we will analyse the effect of the reconstruction error on association analyses in the case-control setting. For this purpose, the simulations will be expanded.

P1046. Probing The Population History Of The R408W Phenylketonuria Mutation Lineages In Europe

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R408W, the predominant Phenylketonuria mutation in Europe, occurs in association with two distinct Phenylalanine Hydroxylase (*PAH*) haplotypes (R408W-2.3 and R408W-1.8) as the result of recurrent mutation. R408W-2.3 shows a west to east clinal distribution across Europe while R408W-1.8 is largely confined to north western Europe (particularly Ireland). Spatial autocorrelation analysis of the distributions of these lineages in Europe has yielded correlograms consistent with dispersal by human migration for R408W-2.3 and with isolation by distance for R408W-1.8. This suggests that R408W-1.8 may have arisen in Ireland, independent of the R408W-2.3 lineage. To further investigate their history, we have documented genetic variation associated with both lineages using VNTR cassette sequence variation, SNP markers from the extended *PAH* haplotype proposed by Zschocke & Hoffman (Hum Genet 1999; 104: 390-398) and three novel dinucleotide STR markers (www.pahdb.mcgill.ca). DNA samples (n=141) were obtained for this purpose from thirteen European regions with appropriate local ethical approval. VNTR cassette sequence diversity has identified different evolutionary histories for the underlying haplotypes (Tighe et al., Hum Mutat 2003; 21: 387-393). A preliminary analysis of STR haplotype diversity associated with R408W-1.8-b5 and R408W-2.3 has allowed us to estimate ages for both mutant lineages of the order of 250 to 400 generations.

P1047. Genetic structure of Armenian population from paternally inherited markers

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Armenia occupying an area of great interest in relation with theories of early human population expansion and language development; a study of Armenian gene diversity and genetic differentiation between regions will provide insight into demographic events.
The mDNA and Y-chromosome are particularly informative for studies of recent migrations because their sequence variants remain associated with each other in total linkage disequilibrium. The human Y-chromosome DNA variation is then an informative haplotypic system for the reconstruction of recent human population history through paternal lineage.
We examine the distribution of Y-chromosome variation in 7 Armenian populations (Karabakh, Lori, Shirak, Syunik, Sevan, Ararat and Western Armenia) using selected 16 biallelic markers (indels and SNPs) within the nonrecombining portion. High-resolution haplotypes were generated for 486 Y-chromosomes.
Seven Y-chromosome haplogroups (Hg) have been identified. The haplogroups identified in our population displayed differences in their frequencies: Hg 1 (0.325), Hg 2 (0.239), Hg 3 (0.033), Hg 9 (0.298), Hg 21 (0.058), Hg 26 (0.039) and Hg 28 (0.008). One interesting point of our population is the presence of the Hg 9 (SNP - M172G) have never mentioned before in the enlarged samples in Armenians. Genetic differentiation reveals differences between the regions. The highest frequency of Hg 1 is observed in the region Karabakh - 0,4143 (Weale et al.: 0,4279). The frequency of Hg 2 is high in Karabakh and Syunik - 0,2713 and 0,2777 respectively.
Further investigations and characterisation of microsatellite repeats will fill up and enlarge general conception about genetic diversity of Armenian population.

P1048. Population genetics of two Romanian population groups and their European genetic relationships based on the nuclear polymorphic markers

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We characterized two population groups from Ploiesti and Bukarest in Romania with nuclear polymorphic markers (Minisatellite of the ApoB-Gene, Minisatellite D1S80 and Microsatellite in the Von-Willebrand-Factor Gene). Further we investigated the extent of genetic influences of European
neighbor populations on the Romanian gene pool. Genetic distance analysis showed a close genetic relationship with greek populations as well as with Italian population groups. Historically this could be the result of intense trading activities of Thracian tribal groups with greek population groups who established trading colonies at the west coast of the Black Sea during the 7th century B.C. Italian influence ist thought to be the result of the occupation of regions around the Danube river in Romania during the time of the Romanian Empire. Greater genetic distance was found between Romanian and German, Slavic as well as Hungarian population groups.

P1049. Familial marriage and its connection with congenital anomalies

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Because of there is high rate of familial marriage (F.M) in IRAN, we decide to study about to and its effect in congenital disorders such as blindness (B), deafness (D) and Congenital heart diseases (CHD). Work began with providing a special questionnaire for B & F, and research began in B & D schools, for the CHD patients that have had a open heart surgery , dated extract from hospital records of them. In control group from 416 family that was selected Randomized., 91 family about 21.87 percent had F.M.
from 424 patient about 58 percent was resulted from F.M and about

16/5 percent had positive family histories, in blindnees patients, from 93 B, about 54 percent of patient had F.M and 44 percent had positive family histories. in CHD, from 75 patient about 30 percent had F.M.
finally the X 2 test and odds ratio was done, in comparison of D & B with F.M and positive history and control group, result was significance (P<0.001) and odds ratio for B= 4/22 & D = 4/93.
In CHD, the X 2 test is done and F.M effect in CHD was positive (P<0.001) and also odds ratio for this study was 1.6.

P1050. Parental employment status and isolated orofacial clefts in Hungary

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Objective: To study the role of parental employment status as indicator of socio-economic status in the origin of isolated orofacial clefts (OFC) and in the use of periconceptional folic acid/multivitamin supplementation.
Material and methods: 1,975 cases with OFC (1,374 cases with cleft lip ± palate and 601 cases with posterior cleft palate), 38,151 population controls without any defects and 20,868 patient controls with other defects compared in the population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities, 1980-1996.
Results: The proportion of professionals and managerial was lower, while the proportion of unskilled workers, housewives and others was higher in the mothers of cases with OFC compared with the population control group. However, the comparison of OFC and patient control groups did not show any difference in the employment status. A lower folic acid supplementation occurred in the professional and skilled mothers of cases with OFC compared with the population control. This difference was confirmed in the professional group of mothers with OFC compared with patient controls as well. Multivitamins were used rarely in the study groups, less frequently in the groups of other mothers with OFC.Conclusion: The prevalence at birth of OFC shows a slight lower employment status as indicator of socio-economic status in the mothers at the comparison with population control group. The higher maternal education do not go together with a higher proportion of folic acid supplementation in the group of OFC.

P1051. DNA loop organization within a region of human chromosome 16q22.1

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The DNA in eukaryotic cells is organized into loop domains that represent basic structural and functional units of chromatin packaging. In the present study, fluorescent in situ hybridization to extracted interphase nuclei has been used for direct visualization of the packaging of a region of human chromosome 16q22.1 containing the LCAT gene cluster. The extremely dense organization of genes within the cluster and its conservation throughout 90 million years of divergent evolution between human, mouse and pig, may indicate that the tight organization of this gene cluster has biological significance. A pool of large-insert bacterial clones from the previously generated 2.8 Mb high-resolution integrated map surrounding the LCAT gene cluster (Frengen, Rocca-Serra, Shaposhnikov, et al., Genomics 70:273-285, 2000) has been used for in situ hybridizations. The results indicate that the whole large area of genomic DNA containing the LCAT gene cluster may be attached to the nuclear matrix. This would show a unique example of such an extended area of attachment, and would further support an assumption that the LCAT gene cluster represents a functional unit of the genome.

P1052. The Iranian human mutation database

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As the number of disease causing genes is rising, it is vital for each country to create a database, enabling them to promote diagnosis, treatment and basic research for these disorders. Iran with the nearly 70 million population, different nationalities, tribes and religions has a highly heterogeneous gene pool and mutation spectrum. We have designed a database in order to facilitate accessing to such a vast resource of genetic data. The Iranian human mutation database (www.IHMD.hbi.ir) is the collection of the information about published or submitted mutations and related polymorphisms from the Iranian population. Up to now, data from more than 30 genetic diseases with distinct genetic loci has been collected with the information about mutation(s) or polymorphisms of each gene locus. References and authors are also listed on the basis of each mutation.
This database provides a valuable source of information for research and diagnostic purposes.

P1053. A new massively parallel SNP genotyping system using oligonucleotide ligation assay/PCR and capillary electrophoresis detection

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We have developed a high throughput SNP genotyping system that offers speed and flexibility, produces reliable, high quality data and supports large-scale genotyping projects for disease research, including association and linkage analysis. This SNPlex™ Genotyping System is based on multiplex OLA/PCR and capillary electrophoresis for high throughput genotyping. The assay uses an optimized universal set of ZipChute™ reagents for accurate read-out on high-throughput capillary electrophoresis platforms. Genomic DNA is interrogated with multiplexed sets of ligation probes targeting currently up to 48 specific SNP loci in each reaction. Such a multiplex reaction utilizes less than 1 ng of gDNA per SNP genotype. A pair of universal PCR primers amplifies all ligation products in a multiplex simultaneously. Amplicons containing internal universal cZipCode™ oligonucleotide sequences are hybridized to a corresponding mix of universal fluorescent ZipChute reagents. ZipChute reagents contain sequences complementary to the cZipCode oligonucleotide sequences and exhibit unique pre-optimized mobilities during electrophoresis. Genotypes are determined by identification of specifically hybridized ZipChute reagents that are eluted and identified by capillary electrophoresis and subsequently associated with target SNPs using the GeneMapper™ Analyzer Software. The time required for electrophoresis is 15 minutes, yielding a throughput of more than 4,600 genotypes per run, or approximately 440,000 genotypes per day on an Applied Biosystems 3730xl DNA Analyzer. Statistics for 1,250 SNPs are presented including the *in silico* assay design, conversion rate, accuracy and call rate.

P1054. Development of an Internet-based database for genetic association studies

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We have developed a database service for the Neuropsychiatric Genetics Unit at UWCM. It stores and analyses data for association samples for a variety of phenotypes: Schizophrenia, Bipolar Affective Disorder, Autism, Alzheimer's Disease and Dyslexia. Currently there are more than 2000 cases, and more than 2000 unrelated controls and 1200 parent-offspring trios. Different phenotypic variables for each sample, and all our data from individual genotyping are stored in a relational database. Information on the markers and genotyping assays is also available.
The main purpose of the database is to assist the work of a number of research groups by providing a convenient access to, and manipulation of data, and by performing some common statistical analyses. The service is powered by MySQL server, application server and HTTP server, and is accessible through the web. The

application is scripted in VisualBasic and JavaScript. All modules for statistical analysis are developed in house using either original or published algorithms.
The following functions have been implemented so far: manipulation of phenotypic data; automated import of genotypic data; customised data output in several standard file formats; case/control Chi2 test; allelic and genotype frequencies with HWE statistics; TDT; LD measures from biallelic markers with the option of using the EM algorithm; error checks on the genotypic data. The analysis is performed in real time.
The database was designed following widely accepted conventions and file formats and the application scripts can easily be adapted to serve other research groups. The example service can be accessed at http://w011.pc.uwcm.ac.uk/example_db/default.asp

P1055. Design of optimal primers for SNP genotyping assays based on primer extension

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A number of assays for high-throughput genotyping of SNPs are based on primer extension. In our own work with designing primers for SnapShot™, extension of fluorescently labelled primers, and Fluorescence Polarisation, we observed a number of primers that did not extend well. We frequently noticed that such primers had low minimum free energy of the secondary structure of the primer, low melting temperature, or both. In order to assist our work, we developed our own programme for primer design. The input is a flanking DNA sequence with the SNP in brackets, e.g. [a/g], and the output is a list of the sequences of all possible forward and reverse extension primers for given parameters, their main characteristics (dG, Tm, GC content) and an image of the secondary structure. The algorithm for the imaging was designed by us.
In order to estimate the degree to which the various primer parameters influenced the efficiency of the extension reaction, we analysed the performance of 131 primers used in our laboratory. The following linear combination of the two quadratic terms (dG and Tm) and GC: 0.845*(1-1/36*dG2)+0.385*(1-0.00135*(Tm-80)2)-0.0063*GC, provided the best fit to the data with R2= 0.40. Other factors are likely to have influenced the results, such as amount and quality of PCR template, quality of primers and operator inconsistencies. The above formula was used to rank the suitable primers according to the their probability to extend efficiently. This ranking might be different for different assays.
The programme is freely available at http://m034.pc.uwcm.ac.uk/FP_Primer.html.

P1056. Human artificial chromosome expression marker: One can't have both, early detection and stable transfer

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We recently microinjected an CMV/EGFP cassette into nuclei of HT1080 and other cell types and consistently found that >1.000 copies are required to detect green cells under the microscope (ref). Here we address the question whether lipofection of the cassette present on large human artificial chromosome constructs (150 kb) results in early green cells and whether these form stable clones. A PAC based, telomerized alpha satellite array of chr. 5 (125 kb) containing a BS resistance marker and the EGFP cassette was lipofected into HT1080 cells. Green cells were marked and fluorescence monitored under BS selection for 20 days. Within the marked areas containing early green cells (n=41, 2 experiments), only one viable colony has formed and 40 areas contained green cells that ceased growth at the 1-8 cell stage. In contrast, outside of the marked areas 16 stable, uniformly green colonies have formed till day 14. These stable clones were detectable by expression of the GFP marker not before day 4-6 post-transfection. FISH analysis of two stable lines demonstrated formation of stably segregating HACs without integration. There is a striking inability of early green cells (presumably high copy transfectants) to form stable clones, whereas cells which are undetectable before day 4-6 (presumably low copy) can form viable clones which subsequently become green. The result suggests that low copy transfer supports stable clone and HAC formation.
ref : Schindelhauer D., Laner A. Visible transient expression of EGFP requires intranuclear injection of large copy numbers. Gene Ther. 2002 Jun; 9(11): 727-30

P1057. Mutation in the gene encoding Lysosomal Acid Phosphatase (*Acp2*) causes cerebellum malformation in mouse.

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In the present study, we report a novel (autosomal recessive) spontaneous mutation named *nax* in mice. *nax* mice exhibit delayed appearance of body hair and display severe growth retardation during development, they also show ataxia like phenotype. Histological analyzes of adult brain revealed an overall impairment of the cerebellar cortex. The classical cortical cytoarchitecture is completely disrupted, the inner granule layer (IGL) is not obvious, the Purkinje cells are not aligned as a Purkinje cell layer (PCL) and Bergmann glias are not spanning the molecular layer (ML). Studies on P0 stage cerebella showed that milder form of phenotype was already apparent at this stage. A genome-wide linkage scan enabled us to position *nax* locus in the middle of chromosome 2, within a region of 800 kb. We identified only a single missense mutation (Gly244Glu) in Acp2, which encodes an orthophosphoric monoesterase of the endosomal-lysosomal compartment with ubiquitous expression; however expression in the brain and testis is considerably higher. The Glu244 mutation does not affect the stability of Acp2 transcript however renders an inactive enzyme. Ultrastructural analysis of adult *nax* cerebellum revealed large lysosomal storage bodies in nucleated cells however analysis on P6 stage cerebellum showed only mild form of lysosome storage, thus suggesting that *nax* phenotype is due to progressive cerebellum degeneration. Identification of Acp2 as the gene mutated in *nax* mice provides a valuable model system to study the role of Acp2 in cerebellum development.

P1058. Linkage disequilibrium in the interleukin-1 (*IL1A-IL1B-IL1RN*) gene complex: extended haplotypes identified by induced heteroduplex generator (IHG) technology.

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Interleukin-1 (IL-1) signalling via the IL-1 receptor type 1 (IL-1RI) is an important pathway in inflammatory processes and the immune response. Genetic polymorphism in the genes encoding IL-1, its receptors, and receptor antagonist, influences the immune response in a number of human conditions, including rheumatoid arthritis, osteoarthritis, periodontitis and insulin-dependent diabetes mellitus. Few studies, however, have examined the influence of extended haplotypes within the region.
The IL1A-IL1B-IL1RN gene complex is part of a large complex of interleukin-1-related genes on chromosome 2 mapping between bases 102250000 and 114000000 (UCSC July 2003 Assembly). These include IL1R2, IL1R1, IL1RL2, IL1RL1, IL18R1, IL1A, IL1B, IL1F7, IL1F9, IL1F6, IL1F8, IL1F5, IL1F10 and IL1RN. We identified genotypes at 8 polymorphic loci across the IL1A-IL1B-IL1RN region, in a cohort of 180 healthy Caucasians. We then determined the extent of linkage disequilbrium (LD) and the distribution of extended haplotypes, using PHASE-based haplotype modelling. We utilised 6 novel "induced heteroduplex generator" (IHG) genotyping reagents for the following single nucleotide polymorphisms (SNPs): IL1A -889C>T, IL1B exon 5 +14C>T, IL1B -31C>T, IL1B -511G>A, IL1RN IVS3 +59A>T, IL1RN exon 4 +72T>C. Analysis of these SNPs, together with a TTA repeat in IL1A IVS4 and an 86bp VNTR in IL1RN IVS2, showed that there was considerable linkage disequilibrium across the IL1A-IL1B-IL1RN region. We identified a total of 37 extended haplotypes, of which 9 showed haplotype frequencies

greater than 0.05. The simplicity and unequivocal nature of the IHG methodology will greatly assist in forthcoming disease association studies within the IL-1 region.

P1059. PHF5a interacts with two ATP-dependent helicases mDomino-s, Ddx1 and splicing proteins U2AF1 and SRp40

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PHF5a is a highly conserved protein from yeast to man. Based on studies in yeast it was suggested that the homologous protein Rds3p in *S. cerevisiae* takes part in the organisation of U2 snRNP particles. By using yeast-two hybrid experiments we could demonstrate that PHF5a interacts with four mRNA processing proteins, namely with ATP-dependent helicases mDomino-s and Ddx1 and with splicing factors U2AF1 and Srp40. Furthermore, using coimmunoprecipitation studies the interaction domain was restricted to the NH_2- terminal part of PHF5a where two putative zinc fingers are localized. In addition, arginine-serine rich regions of splicing factors U2AF1 and SRp40, and glutamine rich regions of Ddx1 and arginine serine glutamine rich region of mDomino-s were identified as interaction domains. Expression of PHF5a and U2AF1 coincided in spermatocytes. Interaction between these proteins could also be detected by *in vivo* coimmunoprecipitation in the spermatocyte specific cell line GC-4. The subcellular localisation of PHF5a using a fusion protein was predominantly detected in nuclear speckles of NIH 3T3 cells and PHF5a colocalised with U2AF1 and Srp40 expression. Therefore, our results indicate that PHF5a resembles a protein which is involved in RNA processing.

P1060. Identification of novel imprinted genes using Quantification of Allele Specific Expression by Pyrosequencing

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A subset of genes in the mouse and human genomes is expressed from only one allele in a parent-of-origin-specific manner. In addition to genes on the X chromosome that are subject to random inactivation of one copy in females, a few autosomal loci are inactivated by imprinting either of the paternal or maternal chromosome. We are aiming at a systematic identification of novel imprinted genes using “Quantification of Allele-Specific Expression by Pyrosequencing” (QUASEP). This is an accurate high-throughput method to measure allele-specific expression differences by analysing exonic SNPs of heterozygous individuals in RT-PCR products. We analysed transcripts from fetal and adult tissues of F1 hybrid mice and humans. We focussed on genes that map to known imprinted chromosomal regions and started with candidate imprinted genes obtained from recent mouse and human microarray studies. Up to now, two putative novel imprinted genes were identified on the basis of monoallelic expression in d11.5 p.c. (C57BL/6J x Cast/Ei)F1 embryos. Both genes show maternal-allele expression and map to imprinted regions on proximal mouse chromosome 2 and proximal mouse chromosome 7, respectively. The human orthologues of these genes are located on chromosome 10p13 and 19q13, respectively. Experiments to confirm imprinting in embryos derived from the reciprocal cross and to verify imprinted expression in adult tissues of hybrid mice as well as in human embryonic and adult tissues are in progress. The newly identified imprinted genes may be good candidates for imprinting-related disease phenotypes on the respective mouse and human chromosomes.

P1061. Analysis of collagen XVIII/endostatin in a bone angiogenesis model

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Stickler, Marshall, and Knobloch syndromes are oculosceletal diseases caused by mutations in *COL2A1*, *COL11A1*, and *COL18A1*. Consistent with the human phenotype, collagen XVIII knockout mice show abnormalities in the eye. However, the bone defect could thus far not be reproduced in mice. Collagen XVIII and its angiostatic cleavage product endostatin which is deleted in most Knobloch patients are prominently expressed in developing vascular mesenchyme. We analyzed collagen XVIII/endostatin in a multicellular in vitro angiogenesis assay using fetal mouse long bones. 17-day-old fetal mouse metatarsals developed PECAM-1, collagen IV and XVIII positive capillary-like structures after 14 days of culture. Outgrowth of endothelial cells was inhibited by addition of recombinant endostatin, but not by mutant non-heparin binding endostatin or the collagen XV endostatin homologue. Although mutant endostatin failed to reduce outgrowth, it still bound to capillary structures while the collagen XV endostatin homologue did not. These data indicate that heparan sulfate dependent interactions are required for the angiostatic function of endostatin while binding of endostatin to endothelial cells can occur in the absence of heparin binding in situ. Furthermore, collagen XV and XVIII derived endostatins differ in their non-heparin binding interactions. Finally, comparison of wild-type and collagen XVIII-/- endothelial cell sprouting from bone explants showed no significant difference in response to vascular endothelial growth factor and epidermal growth factor. These results support a non-angiogenic role for endostatin‘s parent molecule collagen XVIII.

P1062. Developmental and functional characterization of endostatin binding *in situ*

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Knobloch syndrome is characterized by a congenital generalized eye disease and an occipital scalp defect. It is caused by truncating mutations in the *COL18A1* gene on 21q22.3. Collagen XVIII is the precursor of endostatin, a potent inhibitor of angiogenesis and tumor growth. Interestingly, it is collagen XVIII‘s most C-terminal endostatin domain that is either deleted or interrupted in most Knobloch patients. We systematically mapped and characterized endostatin binding sites in murine embryonal development using alkaline phosphatase affinity probes on whole mount embryos and tissue sections. Consistent with the human phenotype, vascular mesenchyme in the developing eye was identified as endostatin‘s primary target. In addition, prominent staining was found in blood vessels of all sizes and epithelial basement membranes while nerve tissues in brain, spinal cord, and spinal ganglia did not react specifically. The endostatin binding pattern overlapped with the immunohistochemical localization of collagen XVIII suggesting that the binding specificity of the entire molecule resides in its most C-terminal domain. Furthermore, a structure-function analysis was performed. Alanine *in vitro* mutagenesis of the prominent heparin binding site within endostatin demonstrated that endostatin binding *in situ* is independent of the molecule‘s affinity for heparin. In contrast, elimination of the heparin binding site in the vascular endothelial growth factor led to complete loss of binding. Finally, inclusion of the collagen XV endostatin homologue into the screen demonstrated striking differences when compared to endostatin which explains the lack of major functional compensation among collagens XV and XVIII.

P1063. Reducing variability and improving workflow in the collecting, transporting, and processing of blood samples for genomic DNA purification using the PAXgene™ Blood DNA System

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Collecting human whole blood samples for genomic DNA purification as part of a multi-center genomic study presents challenges with respect to sample variability and sample processing. The PAXgene Blood DNA System was developed to provide an easy-to-implement, standardized method for collecting human whole blood samples and purifying genomic DNA.

Blood samples are collected in PAXgene Blood DNA Tubes containing a unique additive designed specifically to maintain sample integrity for DNA purification. Collected blood samples can be

stored for up to 14 days at room temperature*, or for longer at lower temperatures, maintaining sample integrity during shipment from remote locations.
PAXgene Blood DNA Tubes are integrated with the PAXgene Blood DNA Kit, which uses a standardized DNA purification protocol. This protocol was developed to minimize the number of procedural steps, streamlining the workflow for DNA purification. DNA is purified using a buffer-based protocol that minimizes carryover of RNA and protein. Throughout the protocol, a single processing tube per sample is used to reduce the possibility of sample mix-up. The purified DNA is of high yield and quality, and is suitable for use in a range of downstream applications.
* DNA yields may be reduced if tubes are stored at 25ºC for longer than 14 days.

P1064. European integrated project on spinocerebellar ataxias (EUROSCA): Pathogenesis, genetics, animal models and therapy

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Twenty two European groups from 9 countries with an excellent reputation of clinical, clinical-genetic and basic research on spinocerebellar ataxias (SCA) have jointly formed an “Integrated project” (IP) to define the pathogenesis and to develop a treatment for SCA patients. The IP is funded by the European Commission and runs from 2004 to 2008. An international standard on the clinical evaluation in form of a “Core Assessment Program for Interventional Therapies of SCA” will be developed based on clinical rating scales, structural imaging, and electrophysiology. The generation of the world largest collection of information on SCA, the European SCA Registry will ensure standardized data acquisition. This powerful tool will facilitate continuous recruitment of SCA patients throughout Europe for linkage analysis, identification of novel ataxia genes and natural history studies. The potential to include all larger European SCA families into linkage analysis will lead to the identification of new SCA loci and to the cloning of novel ataxia genes. Genotype-phenotype correlations will follow. Subsequently, such a combined effort will offer a systematic large-scale search for genetic modifier factors in SCA. EUROSCA will also implement strong research projects to generate and characterize cellular and transgenic models, which will allow a more defined study of the pathogenesis and will serve as a tool for first therapeutic studies. Nine European research groups will be supported by five core facilities such as transgenic Drosophila work, Expression-Chip-Technology, Proteomics, yeast two hybrid technology, and monoclonal antibodies. Training programs will accomplish EUROSCA.

P1065. The role of Mili, a mouse gene of piwi family, in spermatogonia stem cell development and tumorgenesis

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The stem cells for spermatogenesis are single cells in the periphery of seminiferous tubules. The stem cells either self-renew by forming single stem cells or they become interconnected pairs of cells destined to differentiate. Stem cells, pairs, and chains are collectively called undifferentiated spermatogonia, which subsequently become differentiating spermatogonia, spermatocytes, spermatids, and sperm cells. All types of undifferentiated spermatogonia are morphologically and molecularly alike, but they can be distinguished by the absence or presence of synchronized mitotic and apoptotic figures and by their spatial relation to differentiating sperm cells. The deduced amino acid sequences of mouse homologues MILI showed that each contains a well-conserved C-terminal PIWI domain and that each shares significant homology with PIWI. We demonstreted that mili is expressed in primordial germ cells (PGCs) of developing mouse embryos and may therefore play a role during germ cell formation.Mili is specifically expressed in the testis and in mutant mouse of Tfm/y, qk, Ley, olt except W/W^{v}. This means that mili is expressed in spermatogonia as well as in spermatocytes. Interestingly, like testis-cancer antigens, expression of Mili is detected in some tumor cell lines and tumor tissues. In this study we are attempting to determine putative functions for mili. To address functions of mili, we are generating a transgenic mouse under the testis-specific human translation elongation factor-α(EF-1α) promoter. Furthermore, cell lines with an overexpression of Mili is generated and the further phenotypes of this cell lines will be characterized.

P1066. Development and Performance of the VariantSEQr™ Resequencing System in High Throughput DNA Sequence Variation Studies

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The completion of a reference sequence for the human genome and improvements in highthroughput sequencing technology, including the Applied Biosystems 3730xl DNA Analyzer and the BigDye® Terminators v3.1 Cycle Sequencing Chemistry, have enabled the development of the VariantSEQr™ Resequencing System, a fully integrated system capable of quickly resequencing human genes in a cost effective manner. This system consists of PCR primers of known performance, robust PCR and sequencing chemistries and the fully integrated SeqScape® v2.1 software for mutation detection and report generation. We report here results of our development of a validated process for designing primers for high-throughput amplification and resequencing of the promoter regions, exon regions, and flanking intronic regions for genes implicated in cancer and other diseases. Primer design for large scale resequencing projects has been greatly improved by our ability to correlate both unsuccessful PCR amplification and poor quality sequence results to the presence of local and global factors in the genome. Using large datasets of PCR primer amplification results and sequencing data generated during the Applera Genome Initiative, we have developed a model that is predictive of the success rate for a given amplicon and results are being presented here. We will also present data from test sites demonstrating the ability of this system to quickly and accurately detect variations in genes and develop genotypes to help understand the role these variations play in altered function and disease. The validation of this system will permit the resequencing of genes from a number of genomes.

P1067. Community Engagement: The International HapMap Project

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The International HapMap Project seeks to construct a resource for future studies to help find the genetic variants involved in many diseases. Since 2001, the accompanying ELSI issues such as privacy, stigmatization and consent have been discussed. A particular challenge however in population-based studies is how to address concerns about risks and benefits with the populations involved. The HapMap is being developed with 270 DNA samples from four populations: the Yoruba People of Ibadan, Nigeria; Japaneses in the Tokyo area; Han Chinese in Beijing and residents of Utah (USA) with ancestry in Northern and Western Europe (CEPH samples). The need for meaningful community involvement in biomedical research with named populations, while an ethical norm, remains to be realized in practice. Cultural sensitivities include: the identification of specific populations (“labeling”); the potential for undue influence by community leaders or family members; exacerbation of ethnic differences; no direct or immediate benefit; and discrimination and privacy issues. The processes of selecting and approaching communities, for obtaining their input on sampling and for ongoing information and consultation are neither simple nor uniform. Equally important is the need to sensitize genetic researchers, the media and the broader public and international bioethics community about the ethical, social and cultural implications of the HapMap and genetic variation research generally.
This presentation will describe this complex and novel process and offer advise on lessons learned.

P1068. Interactions of y+LAT-1 and 4F2hc studied by fluorescence resonance energy transfer (FRET) microscopy

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y+LAT-1 and 4F2hc are the two subunits of a transporter complex for cationic amino acids located mainly in the basolateral plasma membrane of epithelial cells in the small intestine and renal tubules. Mutations in y+LAT-1 lead to an impaired amino acid transport function of this complex and cause a severe aminoaciduria, lysinuric protein intolerance (LPI, OMIM #222700).
The active transporter is a heterodimer; the subunits, y+LAT-1 and 4F2hc, have been shown to co-localize in the plasma membrane, but the exact process of dimerization is unclear. In this study, we are using FRET microscopy to investigate the interactions of y+LAT-1 and 4F2hc in transfected HEK293 cells expressing y+LAT-1 and 4F2hc fused with fluorescent proteins ECFP or EYFP. FRET was quantified by measuring fluorescence intensity changes in the donor fluorophore (ECFP) after the photobleaching of the acceptor fluorophore (EYFP). An increase in donor fluorescence in cells with an N terminally labelled 4F2hc could be visually detected throughout the cell, from the trans-Golgi network (TGN) to the plasma membrane. This suggests that there are already protein-protein interactions between y+LAT-1 and 4F2hc before they reach the plasma membrane. The physical distribution of the interaction is currently being studied, but the initial results support the theory of 4F2hc's chaperone function in assisting y+LAT-1's transport into the plasma membrane.

P1069. Haplotype structure of the 200 Kb genomic region surrounding human CYP27B1 gene

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There are two key genes in the Vitamin-D metabolic pathway: the 1α-hydroxylase (CYP27B1) and the Vitamin-D receptor (VDR).
It has been reported that variations in VDR are associated with bone mineral density, cancer vulnerability and, susceptibility and progression to several immune related diseases. Nevertheless, no data is available concerning CYP27B1 polymorphisms and those diseases. The aim of our work is to test for association between CYP27B1 polymorphic variants and Vitamin-D related diseases.
Our first approach was to investigate the haplotype structure of the genomic region surrounding CYP27B1. We have used publicly available datasets from 2 human samples. One of them comes from the HapMap-Project and includes genotype data for 18 SNPs which expand 200Kb on chromosome 12, surrounding the CYP27B1. Results show that the analyzed region is characterized by a high pair-wise LD that is indicated by low decay of LD with marker distance and low haplotype diversity. Using available software from http://archimedes.well.ox.ac.uk/pise/ we can define 4 blocks in this region with a maximum of 3 haplotypes/block that account for 90% of all chromosomes in the studied population. In order to get a better insight on CYP27B1 haplotype, we have studied a second sample coming from DNA Polymorphism Discovery Resource. This sample includes genotyping data for a set of 18 SNPs expanding 8 Kb of the CYP27B1. In this region we have defined 3 blocks with 3 haplotypes/block that account for 90% of all chromosomes in this population. Each block can be identified by a maximum of 2 tag SNPs.

P1070. B-box2 protein domain: a novel regulator for protein-protein interactions.

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MID1, mutated in patients with Opitz-BBBG syndrome, encodes a protein that associates with microtubules. The MID1 protein comprises four domains common to a new subclass of the RBCC (RING-finger, 2 B-boxes, Coiled-Coil) protein family together with a fibronectin type III and a C-terminal B30.2 domain. RBCC proteins, like PML and EFP, fulfill central functions in development and disease. We have previously shown that MID1 binds α4, a regulatory subunit of phosphatase2A (PP2A), thereby targeting it towards ubiquitin specific degradation. Most of the known MID1 mutations cluster in the C-terminus and disrupt the interaction of MID1 with the microtubules. Consequently, PP2A can no longer be ubiquitinated and degraded.
To date no specific function could be assigned to any of the B-boxes of RBCC proteins. While we have previously demonstrated that the B-box1 of MID1 binds α4, we have now found that the B-box2 plays an important regulatory role in this interaction. By performing yeast based galactosidase assays we could show that the presence of B-box2 influences the strength of the interaction between B-box1 and α4. Furthermore, point mutations in the B-box2 of MID1 (C195F, from a patient and Q192R, engineered) were analyzed by immunofluorescence experiments after coexpression with α4. Surprisingly, while all three, wild type and B-box2 mutants, bind to microtubules, only the wild type co-localizes with α4. Consistent with these results, a specific function to both B-boxes of MID1 can be proposed. In addition we suggest a novel pathomechanism of Opitz Syndrome in patients with N-terminal rather than C-terminal mutations.

P1071. Genome-wide detection of microdeletions and -duplications in patients with mental retardation by array-CGH

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Mental retardation (MR) is a common non-progressive cognitive impairment (IQ<70) of which the cause is still unknown in more than 50% of the patients. Based on screens of subtelomeric regions it is estimated that the occurrence of subtle gains or losses of genomic regions in patients with idiopathic MR is between 5-10%. In addition, it is well recognized that MR genes are predominantly X-linked. 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 in MR patients. We have constructed a microarray containing more than 4000 BAC and PAC clones resulting in an array with a theoretical resolution of 1 Mb on the autosomes and 100 kb on the X chromosome. Target DNA was produced by DOP-PCR and spotted in duplicate on 3-D slides. The array was first validated by co-hybridization of differentially labeled genomic DNA probes of males and females. The normalized ratio's for the autosomal targets was always close to 1.0 while those for the X chromosome targets were around 0.65. The SD of the replicate spots for each target DNA was below 10%, which proves the reproducibility of the hybridization. Detection of microdeletions was validated with a known 400 kb microdeletion at Xp22. The six target DNA spots within this deletion were clearly discriminated from the other thousands of targets. Our first results from the screening of aneuploids in a selected panel of MR patients will be presented.

P1072. The Retinome – defining a reference transcriptome of the adult mammalian retina/retinal pigment epithelium

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The mammalian retina is recognized as a valuable model system to study neuronal biology in health and disease. To gain a molecular understanding of the underlying processes, great effort has been directed towards the identification and characterization of transcripts with functional relevance in this tissue. With the goal to assemble a first genome-wide reference transcriptome of the adult mammalian retina, referred to as the retinome, we have extracted 13.037 non-redundant annotated genes expressed in the retina/retinal pigment epithelium (RPE) from 27 independent studies by employing a wide range of molecular and computational approaches. Comparison to known retina-/RPE-specific pathways and established retinal gene networks suggest that the reference retinome may represent up to 90% of the retinal transcripts. We show that the distribution of retinal genes along the chromosomes is not random but appears

to exhibit a higher order organization with significant clustering of retinal transcripts at several defined loci, most remarkably on chromosome 17 and 19. The genome wide retinome map offers a rational basis for selecting suggestive candidate genes for hereditary as well as complex retinal diseases and allows in-depth studies of complex molecular networks in the retina that may define normal and pathological states. To make this unique resource freely available we have built a database providing a query interface to the reference retinome (http://www.retinacentral.org/).

P1073. Functional analysis of murine *Foxq1*

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We have isolated a mouse genomic and cDNA clone that belongs to the family of the Fox transcription factors (previously called *HNF-3/forkhead* transcription factors). The 2.7-kb transcript of the murine *Foxq1* gene is expressed prominently in stomach and kidney. Expression of *Foxq1* was detected in stomach and kidney during pre- and postnatal development. In situ hybridisation revealed that the expression of *Foxq1* is located to the base region of the gastric unit. To determine the function of *Foxq1*, we have generated knock out mice by deleting the whole coding region of *Foxq1*. *Foxq1* deficient mice are viable and fertile. No apparent histological abnormalities can be observed in stomach and kidney. In contrast the mice exhibit a silky shiny skin. This silky skin results from a lack of medullary structure in the hair shaft. Radiation-induced mice mutants, called satin mice, exhibit the same phenotype according to the hairs. Satin mice harbour an intragenic deletion in the *Foxq1* gene.

P1074. New centromere-near and subtelomeric rearrangements detected in Pongo pygmaeus supspec.

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The phylogeny of human and ape chromosomes is not yet fully established, although studies on that topic have been done by banding cytogenetics as well as molecular cytogenetics. To refine the established comparative maps probes specific for chromosome-arms, microdissection-derived probes, chromosome bar code, multicolor banding (MCB) or locus-specific YAC/BAC or cosmid probes have been used. For the more detailed molecularcytogenetic characterization of Pongo pygmaeus pygmaeus (PPYp) in comparison to Pongo pygmaeus abelii (PPYa) and Homo sapiens (HSA) we applied a method called subCTM-FISH. 24 chromosome-specific probe sets were created as follows: a whole chromosome paint, the corresponding subcentromeric probes (as described in Starke et al. 2003; Hum Genet 114:51-67) and subtelomeric probes (Knight and Flint, 2000; J Med Genet 37:401-409) are combined in a five-color-FISH approach. Applying all 24 subCTM-FISH probe set, we found new cryptic rearrangements in HSA compared to PPY. HSA has a pericentric inversion in #1 compared to PPY; PPY has a deletion of the subtelomeric probe derived from HSA 17p13.3; the PPY chromosomes homologous to HSA #20 show a co-localization of subcen 20q and subtel 20p, which suggests a possible double inversion. Additionally, hints on more complex events in regions homologous to HSA 1p36.1, #3 and #17 can be suggested. Moreover, PPYa seams to have an additional inversion in the pericentromere of chromosome 1 compared to PPYp. In summary, the systematic application of subCTM-FISH is highly suited to detect up to present overlooked cryptic rearrangements in Hominidae.
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P1075. HAEdb: a novel interactive, locus-specific mutation database of the C1 inhibitor gene

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Hereditary angioneurotic edema (HAE) is an autosomal dominant disorder characterized by episodic local subcutaneous and submucosal edema and is caused by the deficiency of the activated C1 esterase inhibitor protein (C1-INH). Published *C1-INH* mutations are represented in large universal databases (OMIM, HGMD), but these databases update their data rather infrequently, they are not interactive and do not allow searches according to different criteria. The HAEdb, *C1-INH* gene mutation database (http://hae.biomembrane.hu) was created to contribute to the following expectations: (i) help the comprehensive collection of information on genetic alterations of the *C1-INH* gene, (ii) create a database where data can be searched and compared according to several flexible criteria, (iii) provide additional help in new mutation identification. The web site uses MySQL, an open-source, enterprise level, multithreaded, relational database management system. The user-friendly graphical interface was written in the PHP web programming language. The web site consists of two main parts, the freely browseable search function, and the password-protected data deposition function. Mutations of the *C1-INH* gene are divided in two parts: “gross” mutations involving DNA fragments >1 kb and, “micro” mutations encompassing all non-“gross” mutations. Several attributes (e.g. affected exon, molecular consequence, family history etc.) are collected for each mutation in a standardized form. The dataset was the following at the time of abstract submission:

Mutation type	Number of records (%)
Gross mutations	22 (15)
Missense	53 (36.3)
Frameshift	34 (23.3)
Nonsense	13 (8.9)
Splice-site	13 (8.9)
Small insdels	7 (4.8)
Promoter	2 (1,4)
Other	2 (1.4)
Total:	**146 (100)**

This database may facilitate future comprehensive analyses of *C1-INH* mutations and also provide regular help for molecular diagnostic testing of HAE-patients.

P1076. ChIP on genomic clone arrays to study DNA damage checkpoint-mediated response in telomere-initiated senescence

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An increasingly popular application for DNA microarrays is the study of DNA-protein interactions. DNA bound to DNA-binding proteins is cross linked and immunoprecipitated using an antibody against the protein of interest. DNA is then isolated and subjected to fluorescent labelling before hybridising onto DNA microarrays. We have used this application to study DNA damage checkpoint-mediated response in telomere initiated senescence. Senescence is defined as the exhaustion of proliferative potential and can be triggered by telomere erosion whereby proteins such as phosphorylated H2AX, which are usually involved in DNA double-strand break repair, directly associate with uncapped telomeres. To prove the direct association between dysfunctional telomeres and DNA damage response in senescent cells we performed chromatin immunoprecipitation using an antibody against phosphorylated H2AX and hybridised the immunoprecipitated DNA against the original input DNA onto a DNA microarray consisting of large insert clones spaced at approximately 1 Mb intervals across the human genome. By calculating the difference between the ratios (immunoprecipitated versus input DNA) obtained in senescent and quiescent cells we could show that phosphorylated H2AX accumulates at a subset of subtelomeric regions in senescent cells, with preference towards chromosome ends known to harbour short telomeres. Performing the same hybridisation on a chromosome 22q tiling path array consisting of overlapping large insert clones that cover the whole of chromosome 22q not only confirmed the association of phosphorylated H2AX to the subtelomeric region of chromosome 22q, but also revealed that phosphorylated H2AX spreads more than 270 kb inward from the chromosome end.

P1077. ExonPrimer - A Web-based tool for the design of resequencing primers

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The efficient design of oligonucleotide primers for resequencing genomic DNA is crucial to many DNA protocols in the diagnostic and the research laboratory. ExonPrimer is a Perl script that helps to design intronic primers for the PCR amplification of exons. The script needs a cDNA and the corresponding genomic sequence as input. It aligns these sequences using Blat and designs PCR primers to amplify each exon using Primer3. The positions of the exons are deduced from the alignment of the genomic and the cDNA sequences. Insertions/deletions up to 6 base pairs are bridged by postprocessing. The user can influence the size of the amplicons. Exons with small introns in-between are combined. Exons larger than a maximum size are divided into several parts.
Primers are difficult to design when the target region is duplicated or unprocessed pseudogenes are present elsewhere in the genome. ExonPrimer is using gfClient from the Blat software suite to search for these regions and is masking them before primer design. Currently, the search is performed against the July 03 version of the human genome assembly and the mouse mm4 assembly.
Furthermore primers should not be positioned across SNPs. We provide a version of the human genome assembly where all SNP positions contained within dbSNP are masked by a ‚N'. Primer3 can be configured not to design primers across any ‚N'.
ExonPrimer also generates an EMBL formatted sequence file containing exons and intron/exon boundaries. The sequence can be imported as reference sequence into the Staden package.

P1078. Computer and biochemical analysis of homologous to ceruloplasmin sequences in human genome

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Ceruloplasmin (Cp) - copper containing glycoprotein – is one of the key participants of iron transfer through the membranes and transport of extracellular copper. Iron ion transfer through any cell membrane (for example, mitochondrial membranes), is joined with ferroxidase reaction that is catalyzed by Cp-like protein family. Methods of computer and biochemical analysis were used for elucidation of possibility of the existence of Cp-like protein in mitochondria of mammalians. Cp-like sequence was found (accession number NG_001106 (Cp pseudogene)) in NCBI database. Computer analysis of this sequence showed that it can code a potential oxidase of 303 amino acid residues. It contains copper binding motif His-X-His in the middle of a peptide chain and mitochondria delivery signal at N-terminus. Molecular mass of "mature" *pceudo*Cp is about 30 kDa. RT-PCR analysis showed that in the cultured human cells of HepG2 and HuTu 80 cell lines there are products of transcription of Cp pseudogene. At the same time in HuTu 80 cells products of Cp transcription were not found. Cp polypeptides with molecular mass near 30 kDa were found in fractions of mitochondria and inner membranes, obtained from HuTu 80 cells, using immunoblotting methods.
Data presented in the current work unambiguously make us think that in human genome on chromosome 8 there exists one sequence, that contains all the element, typical for the eukaryotic gene of class II. Its supposed protein product is a mitochondrial Cp-like protein. This gene shows activity in some types of cultured human cell lines.

P1079. Brain region specific expression of Ctr1 gene coded putative Cu(I)-importer of mammals

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Disturbance of copper transport results in iron excess into brain cells that lead the development of the neurodegenerative diseases. Perhaps mammalian cellular copper import is realized by putative transmembrane product of Ctr1 gene, which is highly homologous to Cu(I)-transporter of yeast with high affinity to free copper ions. It is paradoxically as in contrast to yeast the most cells of higher eukaryotes contact only with bound copper ions. The analysis of cloned Ctr1 gene structures showed that Ctr1 gene of vertebrates encodes protein with shorter N- and C-ends. Its extracellular N-terminal domain is enriched with Met and His, and cytosolic C-terminal domain contains HCH motif being able to bind copper ions with high affinity. Phylogenetic analysis revealed that Ctr1 genes of all vertebrates form common cluster, quite a distant from the genes of invertebrate and unicellular eukaryotes. *Steady state* Ctr1-mRNA level fixed with RT-PCR strategy shown that Ctr1-mRNA keeping in cortex, cerebellum, hypothalamus, hypophysis, amygdala and hippocamp is almost the same. While, in choroid plexus, Ctr1-mRNA level is three times higher and identical with the liver level. Actual copper concentration in cerebrospinal fluid (CSF) measured using atomic-absorption spectrometry, and amount of copper removed by Chelex-100, and copper ions associated with immunoreactive tissue-specific ceruloplasmin (Cp) were compared. The calculations showed that CSF Cp contains nine copper atoms per one molecule and three-four of them are removed by Chelex-100. The roles of CSF Cp as a putative donor of copper ions and CTR1 as their acceptor in brain cells are discussed.

P1080. Functional evaluation of nucleotide substitutions *in situ* using alkaline phosphatase fusion proteins

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Alkaline phosphatase (AP) fusion proteins have been used for the molecular characterization and cloning of novel receptors and their ligands. Here we applied this rapid, simple, and quantitative technique in order to evaluate the functional significance of two recently identified nucleotide substitutions within the C-terminal endostatin domain of collagen XVIII. While endostatin is deleted or truncated in most patients suffering from Knobloch syndrome blindness, two affected sibs have been reported with a homozygous A179T amino acid replacement within the endostatin domain. This change was not identified in 100 control individuals. A common heterozygous D104N polymorphism within endostatin was found in two further Knobloch sibs and has been associated with increased risk for prostate cancer and leukemia. We generated a set of murine and human AP fusion proteins. Protein production was monitored by AP activity assays and western blotting. The functional equivalence of the highly homologous murine and human AP-endostatin fusion proteins was confirmed using *in situ* staining of E14.5 murine eyes. Moreover, alanine *in vitro* mutagenesis of endostatin's prominent heparin binding arginines resulted in comparably more specific staining patterns with both murine and human mutant AP-endostatin R158/270A probes. Introduction of the A179T substitution into human AP-endostatin and AP-endostatin R158/270A did not impair *in situ* binding. In contrast, the D104N polymorphism showed increased affinity *in situ*. The human AP-endostatin F162/165A fusion protein lacking two unusually exposed hydrophobic phenylalanines as well as unfused AP were used as controls. Finally, binding of AP affinity probes to cultured endothelial cells was quantitated.

P1081. Does the noncoding RNA Reg1d regulate MID1 gene expression?

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Mutations in the MID1 gene cause Opitz BBB/G syndrome, a specific defect of the ventral midline. Although we have acquired detailed knowledge of the MID1 protein function, it remains open how mutations in the ubiquitously expressed MID1 gene can produce a specific ventral midline phenotype. Diverse posttranscriptional and posttranslational mechanisms were postulated.
To shed more light on this issue, we compared genomic sequences of the human and the murine *MID1* gene. Interestingly, we found a

region spanning 1,126 bp in intron 1 of the MID1 gene with 84.5% homology between man and mouse (Reg1d).
Though RT-PCR experiments could show its expression, Reg1d does not exhibit any connection to the *MID1* exons. An open reading frame of significant length could not be detected. Interestingly, northernblot analysis showed transcription of Reg1d from both strands, sense and antisense and also showed conserved tissue specificity of Reg1d antisense transcript expression in man and mouse. Moreover, Race experiments indicate conserved 3' ends of Reg1d sense transcripts. Two different mechanisms how Reg1d might regulate the expression of the *MID1* gene and/or of other genes in a tissue specific manner can be postulated: Firstly, Reg1d could block the transcriptional machinery, a mechanism called intragenic pausing. Secondly, Reg1d could be a precursor molecule that is cleaved to several smaller micro-RNAs that, in turn activate the RNAi machinery. Further experiments will show, whether sense- and antisense transcripts are expressed in the same cell and will shed light on the in vivo function of Reg1d.

P1082. Generating a temporal and cell type-specific Adamts-5 transgenic mouse

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Primary Osteoarthritis (OA) is a chronic progressive joint disorder characterized by an imbalance between degradation and regeneration of several cartilage components. Although OA affects nearly 190 million individuals worldwide, no successful medical treatment exists so far. Compressibility and elasticity of cartilaginous tissues is based on a well-defined balance between degradation and regeneration of two major components of cartilage - aggrecan and type II collagen. In case of OA there seems to be a chronic imbalance between degradation and synthesis of these extracellular matrix (ECM) components, leading to a loss of ECM-homeostasis and finally to the loss of cartilage function. ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) have been identified in cartilage and are largely responsible for cartilage aggrecan breakdown. ADAM-TS5 is known as an enzyme contributing to the process of cartilage degradation in arthritic diseases, but its exact role in pathogenesis of OA is not yet understood.
By generating an Adam-ts5 transgenic mouse we want to elucidate the role of this aggrecanase in the development of OA. Aim of the project is to generate a transgenic line with inducible expression of Adam-ts5 in articular cartilage. The tetracycline (Tet)-based strategy utilizes a bifunctional effector-responder-construct, so only one transgenic mouse has to be generated. We expect that Tet-administration induces expression of transgenic Adam-ts5 which subsequently lead to the pathological changes of articular cartilage similar to those observed in human OA patient. The transgenic mouse should provide a valuable model for the study of OA and a useful system in testing new pharmacological targets.

P1083. A homologue of Drosophila P transposons is a commonly expressed gene in the human genome

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A substantial fraction of vertebrate and invertebrate genomes is composed of mobile elements and their derivatives (>45% of the human genome!). One of the most intensively studied transposon families are the P elements of Drosophila, which were thought to exist exclusively in the genomes of two-winged insects. Thanks to the data provided by the human genome project we have identified a P element homologous sequence in the human genome (Hagemann & Pinsker 2001, Mol. Biol. Evol. 18, 1979). This P element homologous human gene, named Phsa, is 19532 bp long and encodes a protein of unknown function (hypothetical protein FLJ23320) with a length of 903 aa.
Screening of a human lambda DASH II genomic library and FISH analysis revealed only one copy of Phsa located on the long arm of chromosome 4. This finding, the absence of terminal inverted repeats, and the length of the gene indicate that Phsa is a stationary sequence. The examination of the tissue specific gene expression pattern showed that Phsa is widely expressed in human.
The gene product of Phsa contains the recently described THAP domain (Roussigne et al. 2003, TIBS 28, 66), which shows similarities with the site-specific DNA-binding domain of the Drosophila P element transposase. THAP proteins are supposed to correspond to a novel family of cellular DNA-binding proteins which are restricted to animals. Further expression profiling analysis as well as protein arrays and protein/protein interaction studies should elucidate the function of the Phsa gene product.

P1084. Infevers: an evolving mutation database for auto-inflammatory syndromes

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The Infevers database (http://fmf.igh.cnrs.fr/infevers/) was established in 2002 to provide investigators with access to a central source of information about all sequence variants associated with periodic fevers: Familial Mediterranean fever (FMF) TNF Receptor Associated Periodic Syndrome (TRAPS), Hyper IgD Syndrome (HIDS), Familial Cold Autoinflammatory Syndrome/Muckle-Wells Syndrome/Chronic Infantile Neurological Cutaneous and Articular Syndrome (FCAS/MWS/CINCA). The prototype of this group of disorders is FMF, a recessive disease characterized by recurrent bouts of unexplained inflammation. FMF is the pivotal member of an expanding family of autoinflammatory disorders, a new term coined to describe illnesses resulting from a defect of the innate immune response. Therefore, we decided to extend the Infevers database to genes connected with autoinflammatory diseases. We present here the biological content of the Infevers database, including the introduction of two new entries: Crohn/Blau and Pyogenic sterile arthritis, pyoderma gangrenosum and acne (PAPA syndrome). Infevers has a range of query capabilities, allowing for simple or complex interrogation of the database. Currently, the database contains 291 sequence variants consisting of published data and personal communications, which has revealed or refined the preferential mutational sites for each gene. This database will continue to evolve in its content and to improve in its presentation.

P1085. High resolution analysis of chromosomal imbalances using Affymetrix 10K mapping arrays

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Chromosomal imbalances are a key feature of solid tumors as well as many hereditary diseases. Thus, the detection and detailed characterization of chromosomal aberrations is of fundamental clinical importance. Starting in the late 1950ies with the detection of numerical aberrations by simple Giemsa-staining, genome-wide analysis of chromosomal imbalances has made an amazing progress, mostly in terms of increased resolution. Until recently, array-based comparative genome hybridization (array-CGH or matrix-CGH), using ca. 3000 spotted bacterial artificial chromosomes (BACs) as template, were the state-of-the-art, allowing the detection of imbalances at 1-2 Mb (i.e. submicroscopic) resolution. A few month ago, Affimetrix has introduced the "GeneChip 10K Mapping Xba_131 Array", initially designed for SNP-genotyping of 11.555 individual genomic loci.
Using DNA from a large variety of human sources (cell lines, solid tumors and blood from patients with yet unidentified structural aberrations), we demonstrate that the Affymetrix (SNP-) mapping chip is also suited to detect genomic gains and losses at 150-200 Kb-resolution.

P1086. A universal reporter system that improves electronic microarray-based mutation detection and its use in routine human genetics laboratory.

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Among recent technological advances, DNA chip devices, which allow automated, high-throughput genotyping, promise to considerably improve the detection capability of single-nucleotide polymorphisms (SNPs) in clinically relevant genes. We took advantage of the Nanochip® Molecular Biology Workstation (Nanogen™) and the recently introduced microelectronic array technology, to develop a detection method suitable for most of human gene mutations. The current Nanogen procedure is very efficient but costly, as it makes use of expensive dye-tagged reporter sequences that need to be purchased for each gene defect. In order to apply this high throughput technique to a plethora of human mutations in an inexpensive fashion, we have devised a universal reporter strategy. This modification of the Nanogen™ procedure was first validated on the robust Factor V Leiden and Factor II Nanochip diagnostic assays, and were then applied successfully to the more frequent Familial Mediterranean Fever (MEFV) mutations. MEFV, Factor V and Factor II genotypes identified with this improved system were totally concordant with results of other genotyping methods (DGGE, SSCP and RFLP analysis). We then extended the use of this inexpensive new tool to the diagnosis of other common genetic conditions (Cystic Fibrosis, Hemochromatosis, pharmacogenetics). Finally, we took advantage of the usefulness of the Nanogen Workstation to quickly determine optimal hybridization conditions to implement inexpensive gentyping assays that can be performed on conventional glass slide microarrays. This provides the opportunity to study large series of mutations or patients simultaneously and opens the possibility to achieve epidemiological studies at reasonable cost.

P1087. Characterization of Scapinin in mouse and human

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Based on human EST markers we have identified the human Scapinin gene on chromosome 20q13.32-q13.33 which is expressed most abundantly in the brain with two 3.0 kb and 2.7 kb sized transcripts. It exhibits a complex 16 exon structure with alternative splicing at multiple sites especially in the 5'region where four different leader exons (named 1A to 1D) were found some of them containing AUGs in frame possibly expanding the proposed 1557 bp ORF starting in exon 2. Sagara et al. found the human Scapinin protein to be associated with structural components of the nucleus in HL-60 cells and showed binding to the catalytic subunit of protein phosphatase-1 (PP1c) thereby inhibiting PP1 activity in vitro. After identification of the corresponding mouse gene on chromosome 2H4, which is highly conserved in genomic organization, we analyzed expression patterns in detail concerning different tissues, various brain regions and throughout development. Expression of murine Scapinin seems to occur predominantly in the brain as well as three major signals of 3.3 kb, 2.9 kb and 2.5 kb were detected using Northern blots. Detailed expression analysis revealed a tissue specific complex expression pattern in the brain as well as a developmental specific pattern during embryogenesis displaying weak signals from day 10 pc and strong signals from day 15 pc onwards to adult mice.

P1088. Mapping of Chromosomal Breakpoints Associated with Congenital Heart Defects using Fluorescence in-situ hybridization (FISH): a bypass for isolation of candidate disease genes

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Congenital heart defects (CHD) represent the most common group of inborn malformations, with an incidence of almost 1% in the population. The aetiology of CHD is complex and in-volves both environmental and genetic factors. Studies of model organisms and isolation of human genes involved in syndromes, which include CHD in the phenotype, have given some insight in the molecular mechanisms behind CHD. However, the number of identified human genes is less than 20 and CHD is presently far from understood. Mapping of breakpoints in disease associated balanced chromosome rearrangements (DBCRs) have been instrumental in the isolation of many disease genes and may be used as a shortcut for detection of genes involved in CHD. For this reason we have screened the MCN database (MCNdb, www.mcndb.org), which at present contains data on more than 100 patients with CHD and DBCR. The Mendelian Cytogenetic Network (MCN) had been initiated in 1995 as a worldwide collaborative study to identify DBCRs.
We have identified three CHD patients with DBCRs of potential interest. The karyotypes of the patients are 46,XY,inv(5)(q34q13)de novo; 46,XX,inv(5)(p13q13)mat; 46, XY, inv(8)(p23q13)mat. Two of the breakpoints, 5q13 and 8p23, have initially been selected for mapping studies: The 5q13 breakpoint is shared among five unrelated CHD cases in MCNdb. The 8p23 breakpoint overlaps with the critical region in deletion 8p syndrome, which includes CHD in the clinical spectrum. The initial results of the mapping studies will be presented.

P1089. Microarray analysis of the DNA repair gene transcriptome in human testes

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Male germ cell development is a complex process that involves stem-cell renewal, meiosis and dramatic reorganisation of the resulting haploid genome. Meiosis is the key process for recombination and reduction of the diploid chromosome set to a haploid one. To date, about 100 genes have been found, mainly in knockout mouse models, to be implicated in spermatogenesis. Many of the genes that play a role in meiotic recombination are also important for DNA damage repair. To identify new genes which are relevant for male meiosis and infertility, we have analysed the DNA repair gene transcriptome in human testes. Microarrays allow monitoring the expression of numerous genes in parallel. To this end, we developed a cDNA chip with approximately 500 genes which are involved in different types of DNA repair and/or cell cycle control, along with 100 control house keeping genes. This customized gene chip is used to quantify the mRNA expression levels in adult and fetal testes. So far we hybridised four adult testes against a fibroblast RNA pool, experiments with fetal samples (weeks 15 to 24 of gestation) are underway. Approximately 350 genes showed detectable expression levels in adult human testes, approximately 50 were expressed differentially. Microarray results were validated with reverse Northern blots. In some cases, the corresponding proteins were immunolocalised in adult and fetal testicular sections to specific spermatogenic cell types. Systematic expression analyses will provide new insights into the genetics of male infertility and help to develop novel diagnostic and therapeutic tools.

P1090. Candidate genes for learning and memory: microarray analysis of mouse hippocampi during acquisition of the Morris water maze task

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The hippocampus is important for the acquisition of new memories. Formation of long-term memory requires changes in the molecular composition of neurons and structural rearrangements of activated synapses. In this study we assess changes in gene expression patterns in mouse hippocampus that are associated with spatial learning in the Morris water maze (MWM) task. To this end we developed a cDNA microarray chip with approximately 300 genes, which are involved in cognitive processes, in particular learning, and memory along with 50 control house keeping genes. This customized gene chip allows us to quantify the mRNA expression levels in dissected mouse hippocampi and to monitor the expression

of numerous candidate genes for hippocampal learning in parallel. C57Bl/6 x FVB mice were systematically trained in the MWM. They were given four trials per day to find a platform submerged in water. Groups 1-4 were trained for 1-4 days, group 5 performed an additional probe trial without platform at the fifth day. In the course of this training mice showed characteristic learning improvements: the latency time to find the hidden platform was significantly reduced, dependent on the number of trials. Total RNAs from hippocampal samples of learning and control mice have been prepared and will be hybridized to the gene chip. Microarray analyes of the learning-associated changes in gene expression will provide new insights into the genes and cascades involved in hippocampal learning. This may have important implications for several conditions in humans, which are associated with long-term deficits in memory and/or learning.

P1091. Functional analysis of *LGI1* gene and *LGI* gene family in the formation of malignant glioma and in epileptogenesis

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Autosomal dominant lateral temporal lobe epilepsy (ADLTE) was the first human idiopathic epilepsy known to be caused by mutations in the *LGI1* gene (*LGI1*, leucine-rich glioma associated gene 1) which does not code for an ion channel subunit. Additionally, *LGI1* was also shown to be a putative tumor suppressor gene involved in the formation of malignant glioma. Considering the high homology and identical domain structure shared by *LGI1* and the other three members of the *LGI* subfamily, *LGI2*, *3* and *4*, we evaluated the relevance of the whole *LGI1* family in gliomagenesis. RT-PCR experiments with 12 glioma-derived cell-lines and a nonmalignant, immortalized astrocytic cell-line (SVFHAS) showed that at least one *LGI* gene is down-regulated in 12 examined glioma cell-lines. Next, we overexpressed *LGI1*, *2*, *3* or *4* by adenoviral transduction of glioma cell-lines LN-18, LNT-229, T98G, U87MG and SVFHAS. Overexpression of *LGI1* results in a significant growth reduction in T98G and LN-18 (cell-lines where endogenous *LGI1* is downregulated) therefore approving its function as a tumor suppressor. Adenoviral overexpression of *LGI3* reduced growth rates in T98G whilst overexpression of *LGI2* or *LGI4* didn't show any significant growth reductive effects. To further study the function of *LGI1* and thus to understand its role as tumor suppressor gene and the etiology of ADLTE, we also performed 2-hybrid experiments and identified two candidate interaction partners of LGI1. Sequence analysis identified one of them as a putative protein phosphatase, whereas the second one is associated with the cellular mitosis apparatus.

P1092. Role of the Fas-associated protein factor-1 in the germ cell apoptosis

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Fas-mediated signalling system participates in the regulation of germ cell apoptosis in testis. The Fas system is a receptor-ligand signalling system in which Fas ligand (FasL) binds to and activates the Fas receptor (Fas) to initiate a cascade of intracellular events that leads to the elimination of the Fas-bearing cells via apoptosis. In the testis, FasL is found constitutively expressed on Sertoli cells, and Fas is localised on germ cells. After the activation of the Fas receptor, several proteins bind to the intracellular domain of the receptor. The Fas-associated protein factor-1 (Faf1) was identified in the Fas-associated protein complex. We have characterised a gene trap mutant mice (line 40) and found that the gene trap vector is inserted in the intron 8 of the Faf1 gene. The Faf1-trapped gene translates a truncated protein that lacks the C-terminal end. This mutation lead to pre-implantation death of the homozygous mutants. Breeding analyses revealed that the transmission of the gene-trapped allele to the progeny is higher than the wild-type allele. Expression analyses revealed that the expression of the Faf1 in testis is restricted to haploid spermatids and apoptotic germ cells. Using real time PCR and immunohistological staining, we found that ratio of wild-type gametes that undergo apoptosis is higher than Faf1 mutant gametes. These results suggest that the trapped Faf1 allele protects the haploid germ cells from apoptotic pathway.

P1093. Transcriptional control of KCC2, a neuronal K-Cl-Cotransporter

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Synaptic inhibition via GABA and glycine is essential for controlled neuronal activity. Mutations in $GABA_A$ and glycine receptor genes have been implicated in idiopathic epilepsy and spastic disorders. As $GABA_A$ and glycine receptors are ligand-gated Cl^--channels, tight regulation of intraneuronal chloride concentration ($[Cl^-]_i$) is essential for synaptic inhibition. Early in embryonal development, $[Cl^-]_i$ is above its electrochemical equilibrium. Hence $GABA_A$ and glycine receptor activation leads to chloride efflux and depolarization of the cell. This excitatory action of GABA is deemed to be important for evolving neuronal networks. During neuronal maturation $[Cl^-]_i$ decreases with ongoing expression of the neuronal K^+-Cl^--cotransporter KCC2. Finally, GABA ensues the influx of Cl^- and thus hyperpolarization, the correlate of synaptic inhibition. The molecular mechanisms implicated in the unique, temporally and spatially controlled expression of KCC2 are not known, though it has been speculated that a neuron-restrictive-silencer factor (NRSF) binding site in intron 1 of *KCC2* is involved. We designed a novel transgenic approach to characterize the activity of the KCC2 promotor *in vivo*. We cloned a 5'-genomic *KCC2* fragment into a specially designed vector to drive a beta-galactosidase cassette. Transgenic animals were established and analyzed at various developmental time points for reporter gene expression, mimicking endogenous KCC2 expression. We show that intron 1 containing a presumed NRSF-site is dispensable for neuron-specific expression. Deletion of exon 1 does not alter the tissue specificity of the promotor. We currently extend our studies by the analysis of deletion constructs to identify sequence elements essential for CNS-specific expression.

P1094. Familial renal cell cancer: high-throughput translocation breakpoint cloning using chromosome flow-sorting and arrayCGH

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Renal cell carcinomas (RCC) occur in both sporadic and familial forms. The best known example of a familial RCC syndrome is the Von Hippel Lindau cancer syndrome. In addition, RCC families segregating constitutional chromosome 3 translocations have been reported [1]. The list of these latter families is rapidly expanding. We have initiated a survey of Dutch families known to segregate chromosome 3 translocations. Among these, six novel RCC families were identified. One of these families, harbouring a t(2;3)(q35;q21), was studied in detail and two genes were identified that are disrupted by this translocation (DIRC2 and DIRC3 [2,3]). Despite the recent completion of the human genome project, however, the identification of such translocation-associated genes has remained laborious. Therefore, we developed a novel rapid procedure to map translocation breakpoints in detail using flow-sorted chromosomes in conjunction with array-based comparative genomic hybridisation (arrayCGH [4]). Next to a genome-wide BAC array [5], we generated a full-coverage chromosome 3 array for the present study. By employing these novel tools, various familial RCC-associated translocation breakpoints could be mapped to the BAC level in a single-step procedure. Details of this study will be presented, including the identification of novel candidate genes.

[1] *Geurts van Kessel et al., J. Natl. Cancer Inst. 91: 1159;1999.*
[2] *Bodmer et al., Hum. Mol. Genet. 11: 641; 2002.*
[3] *Bodmer et al., Genes Chrom. Cancer 38: 107; 2003.*
[4] *Veltman et al., BioTechniques 35: 1066; 2003.*
[5] *Vissers et al., Am. J. Hum. Genet. 73: 1261; 2003.*

P1095. Interaction of ARHGEF6 with CAPNS1 suggests an involvement of ARHGEF6 in integrin-mediated signaling and cell spreading

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Proteins of the Rho GTPase family are key regulatory molecules that link surface receptors to the organization of actin cytoskeleton. These small GTPases (Rac1, Cdc42, and Rho) are also crucial for neuronal morphogenesis and connectivity. Remarkably, various forms of X-linked nonspecific mental retardation (MRX) have been shown to result from mutations of different components of the Rho GTPase signaling pathway. We found that mutations in *ARHGEF6*, encoding a Rac1/Cdc42-specific guanine nucleotide exchange factor, are implicated in MRX. We used the coding region of *ARHGEF6* as bait in yeast-two hybrid screens and identified CAPNS1, the small regulatory subunit of both m- and mu-calpain as novel binding partner. The interaction was confirmed by co-immunoprecipitation and GST pull-down. The protease mu-calpain has been implicated in integrin-mediated reorganization of the actin cytoskeleton as well as in cell spreading. By replating CHO-K1 cells on fibronectin to induce integrin-dependent cell spreading, we found that ARHGEF6 co-localized with CAPNS1, integrin-linked kinase, and beta1-integrin at the leading edge of actively spreading cells. Inhibition of calpain caused a marked reduction in cell spreading suggesting that this integrin-dependent process is mediated by calpain. Expression of ARHGEF6 in the presence of calpain inhibitors still allowed the cells to spread suggesting that calpain acts upstream of ARHGEF6. Taken together, we suggest that CAPNS1 provides a link between integrin-mediated signaling and activation of Rac1/Cdc42 through ARHGEF6. It is tempting to speculate that the process of cell spreading in fibroblasts is similar to the sprouting and extension of neurites during neuritogenesis.

P1096. Organization and expression of hamster tspy

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TSPY, encoding the testis-specific protein, is conserved in placental mammals and its expression is restricted to the testis. TSPY is expressed almost exclusively in spermatogonia and topology and timing of TSPY expression, suggesting a role in spermatogonial proliferation. Since it was first discovered in humans, TSPY orthologous gene families have been subsequently characterized in many other mammalian species including primate, artiodactyl, perissodactyl and rodent lineages. In contrast to the situation in cattle and primates, where TSPY is organized in a moderately repetitive cluster, including functional members and pseudogenes, a strange situation is observed in the rodent order. While tspy is functionally conserved and organized as two copies in the genus Rattus, laboratory mice possess a single-copy pseudogene that is unable to generate a functional transcript. We speculate that TSPY - as well as other Y chromosomal genes without X chromosomal homologs - usually maintains functionality by being repetitious, as the only mechanism available to Y chromosmal genes to escape from the effects of deleterious mutations.
The aim of the present study is to investigate the functional status of tspy in a species of the family Cricetidae, the syrian hamster. The Mesocricetus auratus tspy resembles the human and bovine ortholog in almost all aspects of structure and expression. Hamster tspy is functionally conserved, organized in multiple copies, and testis-specifically expressed.

P1097. The Pax7 gene expression in the developing mouse head

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Pax7 and Pax3 are homologous transcriptional factors playing a key role in myogenic cell determination during development of vertebrates. An exclusive function of Pax3 in the trunk muscle formation was revealed by experiments in the Pax3/Myf5 double knock-out mice, in which all body muscles were absent (Tajbakhsh et al., 1997). However, the head muscles were found to be intact in these mutant mice, suggesting the existence of a distinct myogenic program operating in the head. The possibility for the compensative role of Pax7 in the head muscles was not considered in that study since its expression in the head was not detected at the developmental stage E10.5. Here, we describe the Pax7 gene expression in the developing mouse head (stage E12.5) determined by a high resolution *in situ* hybridization technique. Pax7 is found to be strongly expressed in the eye and ear surrounding muscles as well as in the face structures that may correspond to the future facial and jaw muscles. On the basis of these data one can assume that activity of Pax7 in the head muscle precursors could compensate the Pax3 deficiency in the aforementioned mutant mice, allowing normal head muscle development. Thus, it is possible that in the cranial mesoderm, myogenesis is as similar as in the trunk, but with Pax7 substituting for Pax3.

P1098. Characterization of a human TSPY promoter

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Human TSPY is a candidate oncogene and is supposed to function as a proliferation factor during spermatogenesis. It is the only mammalian protein-coding gene known to be organized as a tandem repeat gene family. It is expressed at highest level in spermatogonia and to a lower amount in primary spermatocytes. To characterize the human TSPY promoter we used the luciferase reporter system in a mouse spermatogonia derived cell line (GC-1spg) and in a GC-4spc cell line, that harbor prophase spermatocytes of the preleptotene and early pachytene stage. We isolated a 1303 bp fragment of the 5'-flanking region of exon 1 that showed significant promoter activity in GC-1spg and reduced activity in GC-4spc cells. In order to gain further insight into the organisation of the TSPY-promoter, stepwise truncations of the putative promoter sequence were performed. The resulting fragments were cloned into the pGL3-vector and analysed for reporter gene activity in the murine germ cell lines GC-1spg and GC-4spc, leading to the characterization of a core promoter (-159 to -1), an enhancing region (-673 to -364) and a silencing region (-1262 to -669). Database research for cis-active elements yielded two putative SOX-like binding sites in the enhancing region and reporter gene activity was drastically reduced when three nucleotides of the AACAAT SOX core sequence were mutated. Our findings strongly suggest that testis-specific expression of human TSPY is mediated by Sox proteins.

P1099. Shortcomings of Real Time PCR in gene expression studies:Suggestions for improved validity and reliability

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Real-time Polymerase Chain Reaction (Rt-PCR) is rapidly becoming the gold standard for quantitative PCR and is widely used in gene expression studies and analysis of microarray data. Concurrent with several reviews (Bustin, 2002; Stuerzenbaum and Kille, 2001) we report significant problems with reproducing and verifying results of gene expression studies after applying Rt-PCR.
Our laboratory is investigating differential gene expression in pancreatic tissue of Nkx2.2 mutant mice at several embryonic stages using DNA-microarray technology (Affymetrix). Target genes were identified and Rt-PCR was performed on cDNA from the same tissues. The results, after correcting for technical variations (triple samples and reaction standard), were normalized to several 'housekeeping genes'.
Results showed marked divergence in expression of internal standards (beta-actin, GAPDH, 18sRNA) in e15.5 samples and significant changes in target gene expression between comparable cDNA populations isolated on different days. To compensate for inaccuracies during the critical reverse transcription step we propose the use of 'designer' mRNA (dmRNA) oligonucleotides, which

can be synthesized commercially with high purity and at known concentrations, to be added as an 'external standard'. The dmRNA sequence can be optimized for Rt-PCR and it should be specific to prevent cross-reactivity with the target genome.In summary, to improve the validity and reliability of Rt-PCR in gene expression studies special consideration must be given to the selection of the target tissue, the proper choice of internal and external standards and control for DNA contamination to minimize errors of laboratory technique. Results of Rt-PCR should be confirmed by RNA *in-situ* studies.

P1100. Biochip Development For Polymorphism Detection Of Biotransformation System Genes

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Large-scale population studies of genetic predisposition as well as the screening of polymorphism associated with an individual drug sensitivity demand to create an effective, precise and fast method for the detection of many mutations simultaneously. From our point of view, the most perspective method to solve problem, is hybridization on oligonucleotide microarrays (biochips). Thus, our goal was to develop and manufacture a biochip for polymorphism detection of biotransformation system genes. The following loci have been chosen: CYP1A1 (C4887A, A4889G and T6235C), CYP2D6 (G1934A and DelA2637), GSTM1 (deletion), GSTT1 (deletion), NAT 2 (S1, S2, S3 alleles) and MTHFR (C677T). We have used multiplex PCR with subsequent hybridization on the biochip as polymorphism detection method. Principal novelty of the approach is that the designed biochip consists of blocks of genes (from 2 up to 5 mutations on each block). Depending on the field of investigation, the researcher can combine blocks at designing the diagnostic biochip for the analysis of concrete disease or a certain physiological status, and also to change genes inside the block. In parallel, some multiplex asymmetric PCR reactions (their number was defined by quantity of blocks) with fluorescence primers were carried out. We mixed products of the reactions and carried out multiplex hybridization on the biochip. Mutations were discriminated by analyzing fluorescence intensities from separate units on the biochip.
By analyzing mutations in the genes CYP1A1, CYP2D6, GSTM1, GSTT1, NAT 2 and MTHFR, we have shown the efficiency of the approach for gene polymorphism identification in human genome.

P1101. Three dimensional analysis of histone methylation patterns in normal and tumor cell nuclei

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Histone modifications represent an important epigenetic mechanism for the organization of higher order chromatin structure and gene regulation. Methylation of position-specific lysine residues in the histone H3 and H4 amino termini has been linked with the formation of constitutive and facultative heterochromatin as well as with specifically repressed single gene loci. Using an antibody, directed against di-methylated lysine 9 of histone H3 and several other lysine methylation sites, we visualized the nuclear distribution pattern of chromatin flagged by these methylated lysines in 3D preserved nuclei of normal and malignant cell types. Optical confocal serial sections were used for a quantitative evaluation. We demonstrate distinct differences of these histone methylation patterns among nuclei of different cell types after exit of the cell cycle. Changes in the pattern formation were also observed during the cell cycle. Our data suggest an important role of methylated histones in the reestablishment of higher order chromatin arrangements during telophase/early G1. Cell type specific histone methylation patterns are possibly causally involved in the formation of cell type specific heterochromatin compartments, composed of (peri)centromeric regions and chromosomal subregions from neighboring chromosome territories, which contain silent genes.

P1102. Evolution of the protein phosphatase genes PPP2R3A and PPP2R3B: faster evolution in the pseudoautosomal region 1.

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Protein kinases and protein phosphatases represent large protein families regulating key processes in cell cycle and metabolism. Due to the combination of several different subunits a large variety of specificity can be obtained. Protein phosphatase 2A is comprised of three subunits, a catalytic, a constant regulatory and a variable regulatory subunit. PPP2R3A and PPP2R3B are two paralogous genes encoding variable regulatory subunits. Both genes are transcribed in two alternative splice forms.
Nucleotide sequence comparison of PPP2R3A and B in several species revealed that PPP2R3B has been evolving much faster than PPP2R3A. This difference may be due to the genomic localization of the genes. In human, PPP2R3B was mapped to the terminal part of the pseudoautosomal region (PAR1) in Xp22.3 and Yp11.32, while PPP2R3A is localized in the central part of 3q (3q22.2-3). The pseudoautosomal region is characterized by the highest recombination rate observed in the human genome, a high over all GC content and an elevated interindividual variability. PPP2R3A, in contrast, has neither elevated GC nor high variability and its genomic region exhibits average recombination rate. PPP2R3B is the first human pseudoautosomal gene with an autosomal paralog where both mouse orthologs are conserved and have been identified. As described for all other human pseudoautosomal genes Ppp2r3b is not localized on mouse sex chromosomes. The 35 % divergence of human and mouse PPP2R3B is extremely high and in the same range as for human/*Xenopus* PPP2R3B. In contrast, the divergence of PPP2R3A is 11 and 28 % in mouse and *Xenopus*, respectively.

P1103. A genomic microarray for mapping iris hypoplasia and Axenfeld-Rieger anomaly on 6p25

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Microarray technology has been important in the detection and definition of many genomic diseases in research, but is yet to be fully integrated as a diagnostic tool. To delineate chromosomal changes with increased resolution and obtain rapid results for several probes we fabricated an array of 260 human genomic clones comprised of PACs, BACs and cosmids, and applied arrayCGH to samples from iris hypoplasia and Axenfeld-Rieger anomaly patients with linkage to 6p25. DOP-PCR was applied to generate amine-modified products from the genomic clones for spotting on to aldehyde-coated slides. CGH of DNA from patient samples and a normal control was performed on the genomic array. In a blind study, we detected clinically relevant duplications and deletions in 19 out of 20 DNA samples (one sample could not be scored due to high background), and discriminated affected from unaffected individuals. The duplication breakpoints in the iris hypoplasia with glaucoma samples and the telomeric deletion breakpoint in the Axenfeld-Rieger anomaly samples were clearly demarcated; the centromeric breakpoint in the Axenfeld-Rieger anomaly samples was beyond the clone coverage on the array. This study has shown that arrayCGH has potential as a rapid and accurate diagnostic tool.

P1104. The TRPS1 transcription factor is associated with promyelocytic leukemia (PML) nuclear bodies through the interaction with the Topoisomerase I binding RS protein (TOPORS)

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The *TRPS1* gene on human chromosome 8q24.1 encodes a

repressor of GATA mediated transcription. Because it is almost unknown how TRPS1 exerts this function, we used a yeast two-hybrid system to identify co-factors of TRPS1.
Using the C-terminal 647 amino acids (aa) of TRPS1 as a bait, 23 yeast clones were obtained, two of which encode aa 930-1006 of the 1045 aa topoisomerase I binding RS protein (TOPORS). We narrowed down the TOPORS-associating region within TRPS1 to the C-terminal 100 aa (1181-1281) by using a yeast *in vivo* β-galactosidase assay. We verified the interaction of endogenous and ectopically expressed TRPS1 and TOPORS in T48D and COS-7 cells by immunochemical precipitation experiments.
It has been described that TOPORS also interacts with the Topoisomerase I and the p53 tumour antigen, and that it is localized in promyelocytic leukemia (PML) nuclear bodies, but a specific molecular function of TOPORS is unknown. We found that the endogenous TRPS1 is located in dot-like nuclear structures, some of which also contain PML protein. Whereas ectopically expressed TRPS1 is homogeneously distributed throughout the nucleus of COS-7 cells, it is only found in dot-like structures when co-expressed with TOPORS. This indicates that TOPORS is involved in the TRPS1-PML association. PML nuclear bodies play a role in a variety of transcriptional regulation processes. Although we could exclude a direct effect of TOPORS on the repression function of TRPS1 in a luciferase reporter assay, it is likely that the PML associated TOPORS-TRPS1 interaction modulates the function of TRPS1.

P1105. Gene-Ontology Analysis Reveals Association of Tissue-Specific 5‘ CpG-island Genes with Development and Embryogenesis

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A key open question in the understanding of the biology of DNA methylation relates to the origin and function of CpG islands. Limited experimental evidence suggests that CpG islands are associated with promoters or replication origins active during early development. In this work we used a genome-wide Gene-Ontology (GO)-based approach to analyze associations between GO terms and the presence of 5‘ CpG islands in human RefSeq genes. Each of the 3849 GO terms for which there was at least one annotated sequence was analyzed with respect to the proportion of RefSeqs with 5‘ CpG islands annotated to the term. We found that several GO terms showed a highly significant association with the likelihood of 5‘ CpG islands being present in genes annotated to that term. In particular, the term *development* showed a highly significantly increased proportion of 5‘ CpG island genes. When tissue-specific genes were analyzed separately, the association between the term *development* and a higher than expected frequency of 5‘ CpG island genes became more significant, and many of the individual descendant terms of *development* as well as the entire subgraph emanating from this term also showed significantly higher than expected frequencies of 5‘ CpG island genes. The association of increased frequencies of 5‘ CpG island genes and terms related to development was strengthened by the finding that tissue-specific murine RefSeqs with 5‘ CpG islands were associated with a proportion of ESTs from embryonic libraries that was twice as high as that of RefSeqs without 5‘ CpG islands.

P1106. Search of candidate genes for cardiovascular malformations in NF1 microdeletion syndrome

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Patients affected by NF1 microdeletion syndrome show a higher incidence of cardiovascular malformations (CVM) than classical NF1 patients.
Using bioinformatic tools, two candidate genes for CVM were identified: JJAZ1 and CENTA2. The following additional genes of unknown function were also included in most deletions: FLJ22729, MGC13061, EVI2B, EVI2A, RAB11-FIP4, HCA66 and LOC114659.
We performed an expression study to identify the involvement of these genes in cardiovascular development. RT-PCR analysis on RNA extracted from 15th week human fetal aortic arch and heart showed expression of EVI2B, EVI2A, HCA66, LOC114659, JJAZ1 and CENTA2 genes. The analysis on RNA from five human fetal tissues (aorta, brain, heart, muscle and liver) from the 16th to 20th week of gestation showed that the above genes are expressed in the analyzed tissues, with the exception of RAB11-FIP4 which is not present in the aortic arch, while EVI2B is exclusively expressed in the brain.
Northern Blot analysis on the same fetal tissues indicated high level expression of JJAZ1 in the heart, while LOC114659 is more expressed in the aorta; CENTA2 and HCA66 genes showed high expression levels in both aorta and heart tissues.
To determine the expression profile during heart embryogenesis, we performed RT-PCR on mouse 8.5 dpc total embryo and mouse 12.5 dpc and 14.5 dpc embryonic heart which showed the expression of the CENTA2, JJAZ1 and HCA66 orthologuos genes. Whole mount in situ hybridization studies in mouse embryos at different developmental stages are needed to clarify the role of these genes in cardiovascular system development.

P1107. Meiotic recombination breakpoint map of the 22q11.2 region

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The 22q11.2 deletion syndrome is the most frequent genomic disorder with an estimated frequency of 1/4000 live births, and includes the clinical entities of DiGeorge syndrome, velocardiofacial syndrome and conotruncal anomaly face syndrome. The majority of patients (80-90 %) have the same deletion of 3Mb that results from aberrant recombination at meiosis between region specific low-copy repeats (LCRs). It has been hypothesized that in other genomic disorders, like CMT1A/HNPP and Smith-Magenis, aberrant recombination leading to deletion is caused by low recombination rates within the affected region. To study this problem in the 22q11.2 deletion syndrome we have constructed a recombination breakpoint map based on 202 informative meiosis in 14 extensive families with a total of 53 informative markers. Although average recombination frequency over the commonly deleted region is similar to that of the chromosome 22 average, breakpoints are not evenly distributed within the 22q11.2 region. We find regions where male or female recombination breakpoints alternatively cluster. Furthermore, our results indicate that one of the LCRs implicated in the 3 Mb deletion, has a high frequency of female breakpoints, while the other shows very little recombination. In addition, in a family with a member affected with a 22q11.2 deletion caused by an interchromosomal recombination, we found a recombination event to have occurred in the previous generation within one of the LCRs.

P1108. Expression patterns of Tet-Off promoter mice in brain.

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The “Tet-Off-System” developed by Dr. H. Bujard (Heidelberg) allows the generation of inducible transgenic mouse models and is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Tetracycline which inhibits the tTA protein. In order to assess whether a specific promoter mouse line is suitable for the generation of a disease model the knowledge of the brain regions in which the transgene will be expressed is indispensable. The expression pattern of the promoter mouse line states whether the transgene will be targeted to the desired brain regions and in which brain regions a phenotype or pathology is to be expected, respectively. For this reason we studied

the expression pattern of available Tet-Off promoter mouse lines with known expression in the brain (Prion protein (Prp) promoter, Ca^{2+}/Calmoduline-dependent protein kinase II (CamKII) promoter). We crossbred these mouse lines with responder mice transgenic for the lacZ reporter gene. The expression of β-galactosidase in brain regions with promoter activity was detected using X-Gal as β-galactosidase substrate resulting in a blue staining. We stained entire mouse brains for an overall view as well as brain sections for a detailed analysis. The visualization of this expression data in a 3-D brain atlas facilitates the future goal-directed generation of inducible mouse models using the Tet-Off-System.

P1109. Chromosomal instability following retroviral gene transfer in mice

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Retroviral vectors are potential mediators in gene therapeutic approaches, but may also induce leukemogenesis. In order to study combinatorial leukemia-initiating events, a replication-defective retrovirus (RDR) encoding the multidrug resistance 1 gene (MDR1) was used to transduce mouse hematopoietic cells that in turn were transplanted into lethally irradiated mice. Within 6 months after transplantation, 8 out of 21 mice developed either myeloid, erythroid or T lymphoblastic leukemia. One aim of this study was to find out whether leukemic clones show increased chromosomal instability or even clonal chromosome aberrations. Spectral karyotyping (SKY) was carried out according to the manufacturer`s instructions (Applied Spectral Imaging). Image acquisition and analysis were performed with the Spectra Cube™ system and SKY View™ software. Clonal chromosome aberrations were found in 2 out of 6 mice investigated. A reciprocal T(1E3-F;9C-D) was seen in one case; a complex rearrangement involving chromosomes 4 and 11 as well as an additional chromosome 9 derived from an unbalanced translocation T(9;18) in the other. The karyotype is described as: 38-40, XX, Der(4)T(4A2;11D), +Der(9)T(9B;18A2), Der(11)T(11pter->11B3-?4:: 4A2-A3->4D3::11B3-4->11D:: 4A2-A3->4A1), -18. In conclusion, the activation of oncogenes by RDR integration may lead to an increased chromosomal instability and to the growth of chromosomally aberrant clones. This finding may help to better understand the role of chromosome aberrations during leukemia progression.

P1110. Functional assays on antibody chips identify key players in tumor angiogenesis

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Angiogenesis is an important prerequisite for growth and survival of tumors. During this process, hypoxia inducible factor (HIF) stimulates vascular endothelial growth factor (VEGF) transcription. By it's binding to VEGF receptor, VEGF is stimulator of Ras proteins and other central signaling cascades, which lead to cell differentiation and proliferation. In our in vitro system HUVEC cells were treated with oxygen depletion (hypoxia) or endostatin, a promising inhibitor of angiogenesis in tumors. By RNA profilings on the Human Unigene RZPD-2 75k cDNA array we were able to identify interesting new markers for tumor angiogenesis. One example is the Notch4 receptor, which decreases angiogenesis(Abdollahi et al., Mol. Cell., in press). For gene expression studies on antibody arrays, protein lysates were labeled with NHS ester-linked Cy3 or Cy5, respectively, and incubated with a test array as well as with the 512 Ab array from BD. We found out that the results concerning cell cycle promoting factors and many other proteins matched very well with the transcription data, which was a strong argument for specific binding of the proteins to the respective antibodies. Moreover, other interesting candidates became visible, which are hardly detectable by RNA profiling experiments. One reason might be the short half-life of the RNAs encoding certain proteins, as, for example, signaling molecules of the phosphatidyl-inositol pathway. Together with phosphorylation studies on antibody arrays which we have started recently, we are convinced that these techniques allow us to get a deeper insight into the molecular processes involved in angiogenesis.

P1111. Intrachromosomal recombination within pericentromeric HSATII repeats, the mechanism underlying the pericentric inversion of the chimpanzee chromosome 18, homologous to human chromosome 16.

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When looking at the chromosome banding pattern of human and the great apes (orangutan, gorilla and chimpanzee), marked similarities are observed concerning the number and overall appearance of chromosomes across the four species. Nevertheless, some differences are visible when human and chimpanzee are compared. These are the fusion of two chromosomes forming human chromosome 2 and nine pericentric inversions.
Here we describe the molecular characterization of one of these rearrangements, the pericentric inversion of the chimpanzee chromosome 18, which is homologous to human chromosome 16. DNA sequence and FISH analysis were used to determine the breaks and the inverted region, involving the 16p11.2 - q11.2 area.
The 16p11.2 breakpoint of this chimpanzee-specific rearrangement is localized in a region containing interchromosomal gene-rich duplications including the creatine transporter gene, and sequences of HERC2 and variable heavy chain immunoglobulin genes, as well as ALU repeats, (AT)-rich repeats, and HSATII sequences. The breakpoints do not affect the protein-coding region of any gene.
We are currently investigating the expression of three genes adjacent to the breaks, which might be influenced by the inversion. MGC34800 and LOC283912 are included in the inverted part of the chromosome, SHCBP1 is localized approximately 150kb away from the breakpoint in the proximal q arm of chromosome 16.
Our findings reinforce the idea that pericentromeric regions are prone to insertion of segmental duplications, inversions and other rearrangements due to numerous repeats and other satellite sequences.

P1112. Segmental duplication associated with the human specific inversion of chromosome 18: further example of the impact of segmental duplications on karyotype and genome evolution in primates

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The human specific pericentric inversion of chromosome 18, which is not found in great apes, was analysed using breakpoint spanning BACs from the chimpanzee and human genome. Sequence and FISH analyses disclosed that the breakpoints map to a inverted segmental duplication of 19 kb, which most likely mediated the inversion by intrachromosomal homologous recombination. The 19 kb duplication that encompasses the 3' end of the ROCK1 gene occurred in the human lineage. Only one copy of this segment is found in the chimpanzee. Due to the inversion, the genomic context of the ROCK1 and USP14 genes is altered in the human lineage. ROCK1 flanks USP14 in the long arm of the chimpanzee chromosome 17, which is homologous to human chromosome 18. This order is interrupted by the inversion in humans. ROCK1 is localized close to the pericentromeric region in 18q11 and USP14 is inverted to distal 18p11.3 in direct neighbourhood to LSAU-satellites, ß-satellites and telomere associated repeats. Intriguingly, USP14 is differentially expressed in human and chimpanzee cortex as well as fibroblast cell lines determined previously by the analysis of oligonucleotide arrays. Either position effects mediated by the proximity to the telomeric region or nucleotide divergence in regulatory regions might account for the differential expression of USP14. Further analyses including tissues from different primates are needed to evaluate the reason for the up-regulation of USP14 in humans. The assignment of the breakpoint to a segmental duplication underlines the significance of the genomic architecture for genome and karyotype evolution in hominoids.

P1113. The establishment of a custom-built 6,000 BAC-clone-array for CGH-analyses composed of a 1Mb-clone-set and additional tumor-specific and genetic disease-specific clone sets allows for a genome-wide screening for deletions, duplications and amplifications with unprecedented resolution

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We have established a high-resolution BAC-clone array consisting of 6,000 BAC-clones. A genomic clone of app. 150 kb was spaced every 1 Mb (Wellcome Trust Sanger Institute 1Mb Clone Set, Fiegler et al. 2003, Knight et al. 2000). In addition, app. 2,000 clones, which have been used by the contributing laboratories for interphase cytogenetic diagnostics of different tumors and human genetic diseases, were also placed onto the array. We will further increase the array's resolution by implementing another 3,500 FISH-mapped clones (WTSI) which will allow for genome-wide coverage of approximately 300-400 kb by the summer of 2004.
By comparing array-CGH-results that were obtained from this BAC-array with data from the UCSF-array we were able to determine BAC-clones that map to a different cytogenetic location than originally thought. FISH-mapping will be performed for these clones in order to identify their correct chromosomal localization.
First results with DNA from several known tumor cell lines, a patient with trisomy 18 and normal sex mismatched controls demonstrated that deletions, duplications and amplifications were correctly detected.
We have started to analyze DNA from primary tumors, and paraffin embedded tumor material and patients with genetic diseases in order to identify disease-specific genetic aberrations.

P1114. Comparative expression analysis of human X chromosomal mental retardation genes in mouse and chicken

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The non-syndromic mental retardation genes, MRX, are attractive candidates to be human speciation genes. According to our working hypothesis these genes were recently recruited to telencephalic function in the mammalian lineage. This should be testable in a species that is evolutionary far related to human and mammals.
For several reasons chicken seems to be an ideal model organism for such comparative analysis. In a first attempt we choose MRX genes that interfere with the metabolism of the small RHO- and RAB-GTPases: ARHGEF6, GDI1, OPHN1, and PAK3. These genes are known to be expressed in mice hippocampal areas. The respective chicken gene probes were first mapped in the chicken genome. As expected, OPHN1, ARHGEF6, and PAK3 map to the short arm of chicken chromosome 4. GDI maps not to the region with conserved synteny to the human X chromosome but to the telomere of chicken chromosome 1p. Expression analysis was done on brain sections from fetal chicken and adult mouse. In the cerebellum, an ancient part of the brain already present in fishes, a similar expression pattern was observed for the four MRX genes in mouse and chicken. The telencephalon developed later and different in mammals and birds. But birds have as well a telencephalic structure that serves hippocampal functions. In this structure no expression of the four analyzed genes was observed. Therefore we propose that MRX genes were recruited to hippocampal function only recently in mammals and do not serve the same function in birds.

P1115. Animal transgenesis for xenotransplantation

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The presence in humans of xenoreactive antibodies directed against swine Gal antigen present on the surface of xenograft donor cells leads to the complement activation and immediate xenograft rejection as a consequence of hyperacute immunological reaction. The graft of genetically modified organ of a swine depleted of α1,3-galactosyltransferase enzyme that is responsible for Gal antigen origin, would be tolerated with simultaneous administration of medicines decreasing other less severe immunological reactions. To prevent hyperacute rejection it is also possible to modify swine genome by human genes controlling enzymatic cascade of complement or modifying the set of donor's cell surface proteins. For this purpose genetic constructs containing inactivated α1,3-galactosyltransferase gene, human CD59, CD55 and CD46 genes controlling complement activation and human gene encoding α1,2-fucosyltransferase enzyme modifying cell surface proteins were prepared. These genetic constructs were transfected into the pig fetal fibroblast using strontium precipitation, lipofection and electroinjection methods. After selection molecular and cytogenetic characteristic of cells with transgene integrated into the host genome were performed. Nuclear transfer of these cells can generate pigs with the appropriate genotype. Supported by SCSR grants 048/P05/2001/03 and 048/P05/2001/04.

P1116. Purification and activity studies of recombinant hGH, TNFα and FeldI proteins produced in eucaryotic and prokaryotic systems

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Major cat allergen (chain 1 and chain 2 of FeldI protein) and human tumor necrosis factor (TNFα) were obtained in *E.coli* system. Human growth hormone (hGH) was produced in mammary glands of transgenic female rabbits with transgene located on long arm of chromosome 7 by FISH analysis. Homozygous rabbit line was established and lactating females produced inactive form of hGH. No effect of expression of hGH on phenotype and behavior of females and offspring was observed. Purification of recombinant proteins was performed using immobilized metal affinity chromatography specific for proteins with histidine tag. Purification of FeldI and TNFα was carried out in denaturing conditions. From 1000 ml culture approximately 13 mg protein for FeldI chain 1, 43 mg of FeldI chain 2 and 11,4 mg of TNFα were obtained. Purification of hGH was performed in native conditions resulting in 1,5 mg hGH from 1000 ml of milk. After purification both FeldI chains, hGH and TNFα were released by hydrolysis with thrombin or enterokinase. All recombinant proteins were biologically active. In cell cultures cytotoxic activity of TNFα and growth promoting activity of growth hormone dependent cells were determined. In the case of FeldI immunological activity against serum of patients allergic to cat was narrowed to subgroup of patients allergic to FeldI protein by surface plasmon resonance. In case of hGH 2D-electrophoresis of proteins isolated from wild type and transgenic animals to measure changes of protein expression pattern was preformed. Supported by SCSR grant 3P05A10323.

P1117. Functional characterisation of GTF2IRD1 a transcription factor deleted in Williams-Beuren Syndrome

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Williams-Beuren syndrome (WBS) is a developmental disorder, occurring in ~1/20000 live births, which is caused by the hemizygous deletion of ~ 1.5Mb on chromosome 7q11.23. Phenotypic features of the disease include a dysmorphic face, heart abnormalities (typically supra-valvular aortic stenosis, SVAS), growth retardation, infantile hypercalcaemia and mild mental retardation alongside a distinctive cognitive profile where verbal tasks outstrip spatial tasks. The only phenotype unambiguously associated with deletion of a gene is, SVAS and the elastin gene. Detailed mapping of the WBS critical region 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 disease phenotype. Members

of this family of genes show a high degree of similarity and contain varying numbers of a helix-loop-helix like motif known as an I-repeat. To investigate the role of the I-repeats in the biological function of one member, *GTF2IRD1*, we carried out *in vitro* DNA binding assays on three distinct enhancer elements using various GTF2IRD1 constructs. We have shown that GTF2IRD1 binds a consensus DNA sequence present in the enhancer elements and that a specific amino acid motif within one I-repeat mediates this binding. *In vivo* luciferase assays show that GTF2IRD1 can act as a repressor of transcription from reporter constructs containing the consensus binding sequences identified. Aberrant levels of protein from this gene are likely to influence expression of a number of downstream target genes that could result in some of the developmental abnormalities seen in WBS.

P1118. Free manipulation and overstretching of genes for direct nanoscopic sequencing of DNA

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The direct analysis of single macromolecular chains at the level of their primary chemical structure like the nucleotide sequence in DNA, represents one of the current challenges in macromolecular and life sciences. With respect to DNA, the method of direct sequencing would open a new opportunity for gene mapping of humans. Different nanoscopic methods are approaching the resolution level required for direct recognition of single bases: scanning tunneling microscopy (STM), tip enhanced Raman spectroscopy (TERS) and their different modifications. However, much less progress achieved in respect to proper arranging of single DNA-chains on solid substrates to prepare polynucleotides for direct non-destructive high speed analysis.
To elaborate proper molecular pattern one need first to prepare a macromolecular array of single DNA molecules, where the different single polymer chains from the sample are first properly positioned with respect to each other on the surface and then stretched and optionally over-stretched, followed by reading of the structure by some nanoscopic multi-arrayed analyzer.
Moreover, resulted “Addressed Molecular Array DNA-Chip” should be finally improved to correct “Molecular lithography” defects (strong deviations from position and linearity).
We report for the first time on new method of the free manipulation (free 2D-shaping) of already deposited (physisorbed) single chosen ss- and ds-DNA-chains on a substrate. Method allows to move chains as a whole, to stretch it, to remove stretching defects and to overstretch ds-DNA helix into two parallel fixed single strands. Method is universal and can be applied for other classes of synthetic and natural polymers.

P1119. TSGA10 gene encodes a major sperm fibrous sheath protein with a myosin tail domain and expressed in embryogenesis and tumorigenesis as well as spermatogenesis

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Cytoskeletal (structural) proteins can be involved in dividing and differentiating cells such as movement of sperm (spermatogenesis), the invasion and metastasis of cancer cells (tumorigenesis) and fetal tissues (embryogenesis). We have cloned and characterized a cDNA encoding mouse TSGA10 gene which mapped to mouse chromosome one. Tsga10 expressed predominantly in testis (and brain), but is also expressed in other actively dividing tissues. This protein has a myosin tail and Ezrin/radixin/moesin (ERM) domains. We have confirmed in vivo the presence of a myosin tail and ERM domain and have examined whether it can bind to actin filaments. Mouse TSGA10 is expressed in late meiosis. The transcript is made in pachytene spermatocytes. The protein is cytoplasmic and synthesized in the condensing spermatids as a 65 kDa precursor. This precursor polypeptide is transported down the flagellum to the principal regions of sperm tail where it is processed by a proteolytic cleavage, and either during or following the cleavage it is assembled into the sperm fibrous sheath as 27 kDa (pro-Tsga10 N-terminus) in epididymis. Additionally, immunoblotting results were confirmed by specific Immunoflurecent localization of the protein to the entire length of sperm tail fibrous sheath. Using IHC and RT-PCR, this gene/protein which expressed predominantly in testis, is also expressed in other actively dividing tissues including developmental stages of embryogenesis (E12 to E18 in mice) and in some cancers like germ cell tumour and acute myeloid leukemia. The results support our suggestion that TSGA10 may be a good clinical marker for infertility and particular tumors.

P1120. The MID1/PP2A complex: a meeting point of the TGFß signaling cascade and the SHH pathway

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Opitz syndrome (OS) is a malformation syndrome of the ventral midline, which is characterized by hypertelorism and hypospadias, craniofacial, gastrointestinal, cardiovascular and genitourinary malformations, brain anomalies and developmental delay. The MID1 gene, mutated in OS patients, encodes the microtubule-associated ubiquitin ligase MID1, which targets PP2A for degradation via the proteasome. Patients suffering from related syndromes such as Greig syndrome (GS) or Pallister-Hall syndrome (PHS) share features typical for OS. Mutations in GLI3, which encodes a transcription factor that acts downstream of sonic hedgehog (SHH) underly both syndromes. Interestingly we could show, that the MID1/PP2A complex regulates the GLI3 subcellular localization and its transcriptional activity, thus providing a molecular basis for the phenotypic overlap between OS, GS and PHS.
Moreover, overlapping phenotypes of OS patients and patients suffering from Mowat-Wilson syndrome , which is caused by mutations in SMADIP1, a downstream effector molecule of the TGFß signaling cascade, propose possible interactions of the MID1/PP2A complex with TGFß. In line, we here demonstrate an imposing downregulation of MID1 after TGFß3-stimulation. Low levels of the MID1 protein supposedly lead to a decrease in PP2A ubiquitination and degradation resulting in high PP2A activity and provoke a dyslocalization of the GLI3-transcription factor. Thus, our data propose a regulation of SHH signaling by TGFß via the MID1/PP2A complex.

P1121. The influence of the amygdaloid kindling in rat on apoptosis-related genes expression in amygdala and hippocamp.

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Kindling is an experimental epilepsy model involving induction of epileptiform activity by repeated ocal electrical stimulation of the brain.Especially amygdaloid kindled are regarded as a model of temporal lobe epilepsy in man. The ralation between kindling mechanism and morfological abnormalities in epileptic focus remains unclear. The neuron death via apoptosis plays a role in this process.The purpose of our study was investigate the bcl-x, bcl-2, cas-1, cas-2, cas-3 and bax genes expression in amygdala and hippocampus during amygdaloid kindling process.The material of our study were Wistar rats received stereotaxic implantation of one bipolar electrode in the right basolateral amygdala. After a post-operative period the stimulation of amygdala was initiated (500µA) every 24h until 10 sequentiql fullykindled stage 5 was elicited. The seizure severity was assessed according to a modified Racine`s scale (1-5), seizure duration was a duration of limbic (stage1-2) and motor seizures (3-5). The expression of apoptosis-related genes in amygdala and hippocampus was analyzed using RNA-se Protection Assay method.Under our experimental conditions the statistically significant changes between investigated groups were no observed.

P1122. An unique Collection of Forebrain genes preferentially expressed in Embryonic Telencephalon

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Human brain develops in a series of critical steps, that must be correctly orchestrated to give rise to a properly formed brain. Disorders associated to alteration on these steps are still orphan of gene identification. The aim of this project is the identification and characterization of new genes involved in the developing mouse telencephalon and potentially associated to human neurodevelopmental disorders.
To gain this aim we began to characterize individual cDNA clones obtained through subtractive hybridization techniques; this library is enriched of genes preferentially expressed in telencephalon at E14.5. Several CNS genes known to be essential in mouse developing telencephalon were identified through the screening of this subtractive library: Dlx2, Tbr1 and Tbr2, Neurod6, Neurog2, BF1. The analysis includes sequencing and mapping of the cDNA clones, bioinformatic sequence analysis, high-resolution expression analysis by mRNA in situ hybridization, identification of the human homologous genes, generation and maintenance of a database. We developed a MySQL interface to handle the generated data, accessible through a World Wide Web. This is an invaluable tool for both the internal data management and the release of information to the scientific community (http://tess.tigem.it). Our approach led us to identify 100 human “candidate” genes on the basis of their map position in critical regions for neurodevelopmental disorders: Holoprosencephaly, Schizencephaly, Lissencephaly, Microcephaly, Epilepsy, Autism and Schizophrenia. About 45% of these correspond to EST of “unknown function”, some are located in genomic sequence. This represents an incomparable collection to be used for identification of novel genes involved in neurodevelopmental disorders.

P1123. Non-invasive evaluation of embryo morphological plasticity by new transgenic gene cassette

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Transgenic technology is important for developmental science, targeted production of pharmaceutical proteins. Here we describe the production and a quick separation of transgenic green embryos for asymmetrical assay. Six-week-old BCF1 females were superovulated by injecting of PMSG and hCG, respectively, at 48-h intervals. The IRES-EGFP fragment from plasmid pIRES2-EGFP was digested and ligated into the pQE-Tri System vector. *In vitro* Green color test was done by transfection of linear gene cassette into CHO cell lines. Transgenic green embryos were available by excising and purification of transgene with QIAEX and microinjection of transgene into male pronulei of out bred BCF1 mice. The incorporation of the transgene was examined by placing 6-8 cells embryos under UV light with 390 nm excitation. Non-invasive selection of transgenic embryos was performed at the stage of preimplantation embryos. All of the green embryos could be classified as green or non-green under fluorescence and the selected embryos were demonstrated 100% of accuracy of the selection. So we report the easy and rapid selection of transgene integrated embryos and efficient production of transgenic embryos using GFP as a reporter of gene expression and a fusion tag that can be co-inject with another interested gene to monitor protein localization within living cells.

P1124. Simultaneous typing of HPV strains by microarray analysis

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We present a method of identifying different types of human papilloma viruses (HPVs) in tissue samples by hybridisation of the variable L1 region of the viruses to an oligonucleotide microarray. To this end, the geniom®one system (febit ag) is being used. Because of its flexibility in the in situ synthesis of the oligonucleotide arrays, the chip design could be continuously changed and optimised on the basis of the experimental results.
While most HPVs exhibit a high degree of homology in many conserved regions of the genome, there is sufficient difference - especially in the L1 region - to distinguish the various HPV types. Our goal was the creation of a chip for the simultaneous detection of all HPVs, whose sequences are published. Initially, we designed oligonucleotide probes of different length (18, 20 and 22mers), which are specific for the respective L1 ORF. In the hybridisations, both the sense and the antisense strands were analysed. Employing 96 oligonucleotide probes per HPV type, we successfully determined the composition of pooled samples showing only an insignificant number of false positive signals. Furthermore, by selecting the 15 best-performing probes for each HPV type, we were able to achieve a very good specificity of the assay even with complex mixtures. Also HPV types that show a very high degree of homology could be typed with high selectivity. Towards a refined diagnostic analysis, we implemented a quantitative analysis strategy and determined the linearity of the system by analysing pooled samples of known composition.

P1125. Characterization of two new deletions in the *Pax6* region of the mouse

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The extensive human *PAX6* allele database has identified patients with isolated aniridia mainly as heterozygous carriers of intragenic *PAX6* mutations leading to premature truncation of the gene product. Patients expressing WAGR-syndrome were identified as carriers of deletions suggesting additional haploinsufficient genes within the region.
In the mouse most intragenic *Pax6* mutations lead to premature truncation of the gene product and heterozygous carriers express aniridia, microphthalmia and anterior polar cataract. The *Pax6*$^{Sey\text{-}H}$ and *Pax6*$^{Sey\text{-}Dey}$ mutations are both large deletions, homozygotes die shortly after implantation and heterozygotes express severe microphthalmia. These results suggest additional gene/genes within the region responsible for survival in early gestational stages or which interact with *Pax6* in eye development.
In the present study we characterize 2 additional deletions, *Pax6*^{11Neu} and *Pax6*^{12Neu}, which will be useful to identify the regions harboring genes responsible for embryonic lethality and eye development. Both deletions are homozygous lethal at the early post-implantation stage and do not complement for this trait. Heterozygous *Pax6*^{11Neu} mutants express extreme microphthalmia while heterozygotes for *Pax6*^{12Neu} express an eye phenotype comparable to an intragenic *Pax6* null mutation. Characterization of the deletions (polymorphic microsatellites, fluorescent in situ hybridisation) delineates the *Pax6*^{11Neu} mutation to a ~250 kb region mostly proximal to Pax6 while the *Pax6*^{12Neu} deletion includes ~5 Mb mostly distal to *Pax6*. Thus, these deletions overlap at least for a gene within the region responsible for early embryonic survival, and differentiate for the region containing a gene responsible for severe eye phenotype.

P1126. A Novel Gene in Human Chromosomal Region 5p13.2: Structure, Expression Pattern and Comparative Phylogenetic Analysis

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We have identified a novel human gene in chromosomal region 5p13, which consists of 40 exons, disseminated over 108 kb genomic DNA and it encodes a putative protein Hubert(Human Uncharacterized But Eventually Reasonable Transcript) of 2325 amino acids. The new gene is highly similar (85 %) to Rattus norvegicus gene - similar to heterogeneous nuclear ribonucleoprotein A1.
The novel gene is located between NUP (nucleoprotein) 155 and

IDN3 genes and is transcribed in the direction from telomere to centromere. The gene has three alternative polyadenylation signals (AAUAAA).
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 by using RT-PCR and Multiple Tissue Northern Blot. The higher sensitivity of RT-PCR makes it possible to detect some transcripts that cannot be detected in Northern blots, and allows to determine differences between tissues over a broader range.
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.

P1127. Asymmetric PCR increases efficiency of melting peak analysis in the LightCycler

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Background. Allelic-discrimination for SNP-genotyping using the LightCycler is a rapid and robust technique. By using home-made reagents, to detect factor V Leiden mutation, inconsistent results and a non-specific melting peak was observed. To solve these problems, systematic analyses with different oligonucleotide concentrations and ratios were carried out.
Methods. Unlabeled and fluorescent labeled oligonucleotides recognizing c.1691G>A mutation of factor V gene were designed. PCR amplifications and subsequent melting analyses were carried out in the LightCycler with various oligonucleotide concentrations and ratios. To monitor the efficiency, calculated area under the peak values were compared.
Results. Area under the peak values increased by 12.5 fold in case of an amplification primer ratio of 1:6.7 compared to 1:1 of forward: reverse primers. Further increases of reverse primer amount resulted in further but non-significant increases at 1:13.3 or larger ratios. By using a complementary hybridization probe set (detecting the same location, but on the complementary strand), a converse amplification primer ratio (i.e. higher amount of forward and lower amount of reverse primer) was necessary for similar results. Similar trends were observed by comparing different PCR reagents and different SNPs (FII c.20210G>A; HFE c.845G>A and c.187C>G).
Conclusion. Asymmetric PCR resulting in the formation of higher amounts of the target strand dramatically increases the efficiency of allelic-discrimination by LightCycler PCR.

P1128. Characterization of *ECM3*, a novel human-specific gene expressed in normal and osteoarthritic cartilage samples

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In the context of an EST-project aimed at identifying genes and pathways involved in bone growth and differentiation, novel transcripts were isolated from a human fetal growth plate cartilage cDNA library. cDNA 86H12, corresponding to a novel gene located on chromosome Xq28, was selected for further analysis because of its restricted representation in EST databases. Northern and RNA dot blot analysis revealed a main transcript of 2 kb strongly expressed in human placenta. Moreover, we could demonstrate high expression levels in normal and osteoarthritic cartilage samples by real-time PCR. We determined 1750 bp of cDNA sequence, which comprise an open reading frame of 1320 bp preceded by a STOP codon. The predicted protein has a length of 440 amino acids with 8 leucine-rich repeats and a signal peptide. Based on amino acid sequence similarity to biglycan, ECM1 and ECM2 we designated the gene *ECM3*. Surprisingly, the gene is not conserved in mouse, although some sequence similarity can be found on the genomic level. The strong expression in specific tissues, the conservation of an open reading frame in human and the structure of the putative protein suggests *ECM3* to be a member of the novel group of species-specific genes. The expression pattern, sequence similarity to biglycan as well as multiple metal response elements (MREs) in the *ECM3* promotor (potentially important in the regulation of chondrogenic gene expression by heavy metals) indicate that ECM3 might be involved in skeletal development and/or homeostasis and thus represents a candidate gene for skeletal disorders.

P1129. EuroBioBank (EBB): European Network of DNA, Cell and Tissue Banks for Rare Diseases

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Rare diseases affect around 20 million citizens in Europe. Due to their rarity and the consequent lack of relevant information, patients affected by rare disorders do not benefit from accurate medical resources similarly to persons affected by more common pathologies. In order to develop a coherent European network for easily accessibility of human biological resources on rare diseases, the EuroBioBank project has been initiated in 2000.
EuroBioBank started to connect scientific Groups, DNA, cell and tissue banks in order to identify and localise biological materials (DNA, tissue, cell cultures) of rare diseases, to harmonise and spread quality banking practices, to distribute quality material and associated data to scientific users, and to disseminate knowledge and know-how to the scientific community through specialised training courses, conferences and articles and a website dedicated to the network activities (www.eurobiobank.org).
Expected achievements arean optimization of existing collections and banks; an improvement in medico-scientific collaborations in the field of rare diseases, the development of new therapeutic methods and specific research and diagnostic tools for rare diseases.
EuroBioBank is a successful, structural model for supporting scientific exchange and cooperation. It was financed through the 5th framework programme of the European Commission (project QLRI-CT-2002- 02769, for 2002 - 2004 years). EBB involves 16 Partners from 8 European Countries (Belgium, France, Germany, Hungary, Italy, Malta, Slovenia and Spain), representing at least 65000 samples of DNA and 15000 samples of tissue in total. The network is open to new partners.

P1130. Inducible expression of human alpha-synuclein in transgenic mice

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The presynaptic protein alpha-synuclein has been implicated in the pathophysiology of many neurodegenerative disorders, including Parkinson´s disease and Alzheimer disease. To advance the study of the function of alpha-synuclein in these diseases, we have used the tTA- system to generate transgenic mice, which express human wildtype or mutated [A30P] alpha-synuclein in a conditional manner. To obtain reversible expression of human alpha-synuclein in the brain of these mice, we combined the tTA-system with both the PrP promotor and CaMKIIalpha promotor. We investigated the conditional over-expression by western blot analysis and immunostaining of paraffin-embedded brains. Western blot analysis revealed that double-transgenic mice express alpha-synuclein at different levels in specific brain regions. The expression-pattern and level of human alpha-synuclein in the brain of double-transgenic mice depends on both the neuron-specific promotor and the integration site of the human alpha-synuclein construct. Histological analysis of transgenic mice showed aberrant expression of the protein in cell soma. However, Lewy body-like alpha-synuclein inclusions have not yet been identified. Administration of doxycycline down-regulates alpha-synuclein expression to basal levels in the brain of double-transgenic mice. We currently investigate if one transgenic mouse-line which highly expresses mutated [A30P] alpha-synuclein specifically in the olfactory bulb shows any loss of dopaminergic neurons and an impaired sense of smell. We perform microarray expression analysis to gain insight in the pathomechanism underlying over-expression of human alpha-synuclein. Our conditional mouse-model may help to define the role of human alpha-synuclein in synucleinopathies and might be used to demonstrate whether neuropathological symptoms of these diseases are reversible.

P1131. Fbx25, a novel brain-expressed F-box protein, binds Cul1 and not Skp1, but its E3 complex retains ligase activity

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We investigated the novel human hFBX25 gene and its highly homologous mouse counterpart, mFbx25. Both genes encode proteins belonging to the F-box protein family. The F-box domain confers substrate specificity to the SCF-type (Skp1/Cul1/F-box protein) of ubiquitin protein ligase (E3) complexes. The ubiquitin pathway is important in a variety of fundamental biological processes and aberrant ubiquitin-mediated protein degradation has been implicated in a range of human diseases, including the neurological disorders Alzheimer‘s, Parkinson‘s and Huntington‘s.
In situ hybridisation with an mFbx25 probe on mouse embryonic sections shows neuronal tissue specific expression. Expression in mouse adult brain is confined to the hippocampus and the cerebral cortex.
In HeLa cells, binding of the overexpressed hFBX25 protein with Skp1 and Cul1 was assayed by immunoprecipitation showing interaction between the hFBX25 F-box and Cul1, but surprisingly not with Skp1. Interestingly, the ligase activity of the Fbx25 E3 complex is not abolished by this lack of Skp1 binding. These findings suggest a novel type of E3 ligase complex. In silico analysis indicates that the inability of the hFBX25 F-box to bind Skp1 is due to the occurrence of a serine residue at position 244 instead of a highly conserved leucine residue. This hypothesis is currently investigated by overexpression studies of an S244L variant generated by in vitro mutagenesis.

P1132. Genetic polymorphism studies in Russian population using biochips

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Gel-based microchips, manufactured by photoinduced copolymerization of oligonucleotides and components of gel, have properties allowing very reliable identification of point mutations. Using of short oligonucleotide probes (15-20 bases long) provides the high level of discrimination between perfect and imperfect duplexes, while immobilization in the volume of gel significantly increases the fluorescence intensities of positive hybridization signals that allows to use relatively simple and cheap fluorescence detectors.
The method of point mutation analysis includes multiplex PCR following by allele-specific multiplex hybridization with biochip.
One example is the analysis of mutations in TPMT-gene encoding cytosolic enzyme, which participates in the metabolism of thiopurines. The designed TPMT-biochip is able to determine most widely spread mutant alleles, encoded TPMT-enzyme with low level of activity.
The large-scale screening is performed in order to determine the frequency of different alleles in Russian population. The identification of genotypes can be done fully automatically using portable analyzer and software. The work is carried out in collaboration with St. Jude Children's Research Hospital (Memphis, USA). The TPMT-biochip is supposed to be used in medical practice to determine individual drug sensitivity. The biochip for the analysis of biotransformation system genes polymorphism, including most functionally significant alleles of CYP1A1, CYP2D6, GSTM1, GSTT1, NAT 2, CYP2C9, CYP2C19, NQO1 and MTHFR genes has been also developed. The biochip is used in the analysis of association between allelic variants and predisposition to oncological diseases. The data demonstrate the possibility of wide use of gel-based biochips in population studies as well as for medical diagnostics.

P1133. Conditional Activaton Of Cre Recombinase Activity In The Kidney: A New Tool To Study Polycystic Kidney Disease

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Conventional gene knockout strategies have resulted in a wealth of developmental, physiological, and pathophysiological information. Despite this, whole animal gene disruption often leads to prenatal lethality therefore precluding analysis of its functional role in adulthood. In addition, determining the biological role of a targeted gene in a specific organ or cell type may be confounded by functional changes in other organs. These issues are particularly relevant to gene-targeting strategies used to examine renal function or to explore the feasibility of gene therapy of e.g. polycystic kidney disease. The kidney contains at least 27 different cell types and it is apparent, that more specific means of gene disruption are necessary to evaluate the role of that particular gene product in a given renal cell type in vivo. To conditionally target kidney proximal tubules we generated transgenic mice expressing a Tamoxifen-inducible CreERT2-recombinase under the control of the truncated form of the gamma-glutamyl-transferase (GGT) promoter. Analyses using Rosa26 reporter mice showed that 1. Cre-recombinase activity can be induced by a single dose of Tamoxifen in adult mice and 2. Cre-recombinase activity is restricted to S3-segments of kidney proximal tubules. Together with previously published Cre-recombinase expressing mouse lines targeting different segments of the nephron (collecting duct principal cell: aquaporin-2 promoter; podocytes: nephrin-promoter; thick ascending limb: Tamm-Horsfall promoter; developing kidney and genitourinary tract, collecting duct: the Ksp-cadherin promoter) this transgenic mouse will provide a powerful tool to study gene function in the kidney and to create various mouse models mimicking human renal diseases.

P1134. Identification of a novel Lim Domains Containing Gene that interacts specifically with Ror2

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ROR2 is a receptor tyrosine kinase, that belongs to a family of receptor tyrosine kinases with a conserved cytoplasmatic kinase domain, distally located serine/threonine-rich and proline-rich domains and distinct extracellular domains, namely an immunoglobulin, a cysteine-rich and a kringle domain. Ror2 has been implicated in chondrogenesis as shown by the severe chondrodysplasia phenotype in Ror2 knock out mice. However, the signalling pathways in which Ror2 participates remain unidentified to date. Mutations in ROR2 have been described to be responsible for autosomal dominant brachydactyly type B and the autosomal

recessive Robinow syndrome .
We used the cytoplasmatic part of ROR2 to perform yeast two hybrid-assays. We phosphorylated ROR2 by co-expression of constitutively active SRC-kinase. In a second set of experiments we used unphosphorylated cytoplasmatic ROR2 as bait. Screening was performed against a cDNA library obtained from mouse embryos stage E9.5 to E10.5. We were successful in identifying several clones that are potential interaction partners. In particular, we identified a novel LIM domains containing protein that was shown to interact with ROR2 in a phosphorylation-independent manner. Interaction could be confirmed by co-immunoprecipitation in hek 293-cells and was located to the distal serine/threonine- and proline-rich domains. In cos1-cells ROR2 and the LIM protein show co-localization. This ROR2 interacting partner is a novel member of the LIM protein gene family. It locates to mouse chromosome 7 and consists of 9 exons. LIM domain proteins belong to a diverse family that share different numbers of LIM domains, which have been shown to mediate protein-protein interactions.

P1135. Alu mediated mRNA-folding cause exon skipping of the Galectin-1 gene

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Alternative splicing is a sharply regulated cellular system originating diverse mRNA forms from a single primary transcript. Multiple mRNAs result from different exon boundaries definition or complete exclusion/inclusion of entire exons. Several sequence variations (i.e.: Alu insertion) can remodel splicing schemes.
The galectin-1 gene (LGALS1), located at 22q12, by our analysis and EST comparison shows skip of the exon 3 with frame-shift and premature stop-codon formation. Gene sequence analysis shows the lack of the exon 3 at cDNA level, without any splice-junction defect and any other variation. Repeat Masker program identify in the second and third intron three Alu insertions, which were assigned to the S family. Zuker algorhytm (Mfold) implementation draws a long match between these introns. The stem-loop structure formation of the primary RNA transcript involves more than 200 bases extended to almost all the Alus length (>83% of complementarity). We suggest that the extended matching between the Alu elements in flanking introns, concealing both splicing sites and exonic sequence, may induced exon skipping.
The proposed model underlines the role of Alu elements to guide mRNA folding which in turn may regulate alternative splicing.
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P1136. Polycystin-2 directly influences cardiac outflow tract development

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Inactivation of the polycystic kidney disease genes type 1 and type 2 (Pkd1 and Pkd2) cause the multisystemic “autosomal dominant polycystic kidney disease” (ADPKD). The prominent characteristics of this disease are large fluid-filled cysts in the kidneys, pancreas and liver. Beside additional malformations, miscellaneous defects occur during cardiogenesis resulting in congenital heart diseases. We generated a Pkd2 knockout mouse model and could not only observe these defects but additionally could show that polycystin-2 is required for left-right axis determination. Loss of function of polycystin-2 resulted in a complete disruption of the nodal pathway causing severe disturbances of the embryonic turning, heart looping and abdominal situs determination. More strikingly is the fact that uncoupling of polycystin-2 is leading to a developmental variation of the cardiac outflow tract as we could observe in heart dissections of different embryonic stages. Beside this conotruncal defects different kinds of heart malformations like VSD and ASD were found which impair the cardiovascular system and are probably the main cause for early prenatal lethality in our Pkd2-/- mice as they die in utero at embryonic day (E)13.5. To dissect genetic pathways relevant to heart development we performed whole mount in situ hybridizations with several important cardiac keyplayers. First hints out of our preliminary experiments point to a correlation of outflow tract malformations with the non-canonical Wnt signalling pathway. Data out of these experiments will be presented.

P1137. Immortalized renal embryonic epithelial cells from Pkd2 knock-out mice

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Mutations of either PKD1 or PKD2 cause autosomal dominant polycystic kidney disease (ADPKD), a syndrome characterized by extensive formation of renal epithelial cysts and progressive renal failure. Homozygous deletion of murine Pkd2, the gene encoding polycystin-2 (PC2) results in prenatal lethality. To study cellular processes including differentiation, polarization, growth, adhesion, signaling, ion fluxes and cytoskeletal organisation in more detail we established Pkd2 -mutant renal epithelial cell lines that carry a temperature-sensitive SV40 large T antigen oncogene (H-2Kb-tsA58) derived from the ImmortoMouse. To establish these epithelial cell lines, kidneys from 16.5 old embryos were digested with collagenase and epithelial cells were enriched with PNA- or DBA-coated Dynadeads. Cells were seeded on collagen coated culture dishes and cultured under standard conditions described for kidney epithelial cells. Cells isolated from control and PC2-deficient embryonic kidneys in culture displayed an epithelioid cell phenotype and expressed E-cadherin and its associated catenins as expected. When seeded on collagen and cultured under non-permissiv conditions, T Antigen expression was down-regulated after 2 days of culture. All the cells expressed lectins Dolichos biflorus agglutinin; only a small percent of the Pkd2-/- Immorto+ cells expressed Arachis Hypogaea agglutinin suggesting collecting tubule origin. We have been successful in isolating immortalized renal epithelial cells which are derived from the collecting duct of Pkd2 knockout and control mouse embryos. These cells lines will allow the identification of gene products differentially expressed and signalling pathways affected in PC2 deficient cells.

P1138. HD transgenic rats and knock-in mice: Microarray analysis and comparison of altered expression networks

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Huntington’s Disease (HD) is an autosomal dominant inherited neurodegenerative disease caused by a CAG repeat expansion. To date, several transgenic mice models have been described that exhibit symptoms similar to those seen in affected patients. Recently, we have developped a transgenic rat model for HD which shows slowly progressive neurological, neuropathological and neurochemical phenotypes closely resembling the common late manifesting type of disease. Polyglutamine induced changes in gene expression have been examined using several mouse models of HD. Their microarray data indicate that there are substantial differences between the various models of HD.
Here we report the first microarray analysis on a transgenic rat model and a knock-in mouse model for HD. RNA for chip-analysis was isolated from the cerebral cortex and the striatum. As controls we used mouse strain C57Bl6 and wild-type littermate controls of the HD transgenic rats. Three 3-month-old and 12-month-old males, respectively, were analysed for each model using RG-U34A and MG-U74A chips. The results were analysed using standard Affymetrix software. Almost 143 mouse and 178 rat genes were found to be either up- or downregulated. Although we found significant differences between both models, there was a considerable overlap in the genes showing altered gene expression. Cluster analysis revealed genes of related function that exhibit similar expression patterns over time.
This time-course study with comparison between two different species will provide new insights and will allow investigators to identify a molecular pathway that proves to be central to the disease process.

P1139. Widespread expression of a novel non-muscle yosin heavy chain gene MYH14

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We could identify a previously unrecognized nonmuscle myosin II heavy chain (NMHC II, MYH14) in human chromosome 19q13.3 (accession nr.: NM_024729). This myosin constitutes a distinct branch of the nonmuscle/smooth muscle myosin II family. The genomic structure analysis predicted 41 exons in about 100kb. A cDNA of about 7kb encodes a polypeptide of 1995 amino acids, calculated 228 kDa. Out of five *in silico* predicted splice variants one could be attested experimentally. This one was not represented in the EST-databases so far. MYH14 shows homology with other non muscle myosins- MYH9 and MYH10 - as well as with a smooth muscle myosin MYH11. However, phylogenetic analysis suggests that MYH14 constitutes a distinct branch of the non muscle / smooth muscle myosin II family. Northern blot analysis demonstrated that MYH14 is expressed in a wide range of tissues mainly in colon, skeletal muscle and small intestine. With the exception of a partial mouse amino acid sequence no complete MYH14-homologous gene or protein was found in other species yet.We performed antisense RNA *in situ* hybridization experiments based on fluorescent labelled oligonucleotides and probes generated by radiolabelled *in vitro* transcription. Semithin crysections of E 13.5 mice were used. MYH14 is strongly expressed in developing alveoles of the lung and in liver cells. Furthermore it is expressed both in the developing and adult brain, in spinal ganglions were also visible signals. The ubiquitous expression suggests a general biological importance of this gene, but yet no disease could be associated with the nonmuscle myosin MYH14.

P1140. The Chicken “Micromass Culture” System - functional analysis of mutations in GDF5 and BMPR1b causing inherited hand malformations

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Primary mesenchymal cells from chicken limb buds are able to differentiate into chondrocytes in vitro. This can be studied in the so called “micromass culture” system. Transfection via an avian retrovirus allows introducing a specific gene of interest. Effects of the expressed genes or mutant variants can be monitored directly by a set of methods analysing the differentiation status of chondrocytes. For example, early chondrogenesis is characterised by a specific matrix production, which can be quantified by Alcian blue staining, whereas mature chondrocytes are identified by their alkaline phosphatase activity. Quantification of marker gene expression by real-time PCR offers the opportunity to follow the changes on a molecular level.

We used the micromass culture system for functional characterisation of mutations in Bone morphogenic protein Receptor 1b (BmpR1b) and Growth and Differentiation Factor 5 (GDF-5) causing different inherited hand malformations.

Our studies revealed that mutations in BmpR1b (I200K; R486W), which cause brachydactyly type A2 (BDA2), have a dominant negative effect on early chondrocyte differentiation, characterised by a reduction of Alcian Blue staining, in the chicken micromass culture system.

Currently, we are analysing mutations in GDF-5, a ligand for BmpR1b, causing either brachydactyly type C (M173V, S204R, R438C) or symphalangism (R438L).

In contrast to the mutations investigated in BmpR1b, first results show that these GDF5 mutations have no inhibitory effect in the chicken micromass culture system.

P1141. TCF4 protein interacts with TWIST and contributes to its nuclear import

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Mutations in the TWIST gene are described to cause Saethre-Chotzen syndrome (SCS; MIM #101400), an autosomal dominant craniosynostosis syndrome. It is characterised by premature fusion of coronal sutures which leads to skull deformation, facial dysmorphisms accompanied by subtle limb abnormalities.

TWIST, a bHLH transcription factor, regulates its target genes as a heterodimer with other bHLH proteins. Besides the well characterised bHLH domain, we identified four additional conserved domains in the human TWIST protein by an evolutionary alignment from *Fugu* to human. Alongside of two putative nucleus localisation signals (NLSs), we detected the functionally unknown NSEE and WR- domains.

The two conserved classical NLS motifs are present at amino acid position 39-41 (NLS1) and position 75-79 (NLS2). In order to analyse the functionality of the classical nuclear localisation signals in more detail, conserved positions of the amino acid sequence in NLS1 and NLS2 were altered by site-directed mutagenesis.

Human U2OS osteosarcoma cells were used for transient transfection with myc-tagged constructs of the TWIST protein and the localisation of the mutated constructs was analysed by immunofluorescence microscopy. The altered TWIST NLS1-construct caused a cytoplasmic relocalisation indicating that the NLS1 motif is functional as a nucleus localisation signal *in vivo*.

In an alternative approach to identify factors that could influence the functionality of TWIST, the yeast-2-hybrid system was used to screen for interacting proteins. Up to now, several promising candidates have been identified, among them members of the class A bHLH transcription factors that include the gene product of TCF4.

P1142. Characterization of the skeletal phenotype of the Ror2 knock out mouse

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Mutations in the receptor tyrosine kinase *Ror2* account for autosomal recessive Robinow syndrome (RS) and dominant Brachydactyly type B (BDB). We have analyzed the underlying developmental pathology of *Ror2*$^{-/-}$ mice, characterized by short stature, mesomelic limb shortening, malformations of the spine, craniofacial malformations and small external genitals. Analysis of somitogenesis indicates that the vertebral malformations in *Ror2*$^{-/-}$ mice are due to a smaller presomitic mesoderm and defects in somite formation and differentiation. Mesomelic limb shortening in *Ror2*$^{-/-}$ mice results from a perturbed chondrocyte differentiation as indicated by abnormal chondrocyte shape and reduction of *Ihh* and *Col10* expression. To further characterize the defects in bone formation and to identify possible *Ror2* target genes, we have performed Microarray analyses using Affymetrix arrays. We compared the gene expression profile of wildtype humerus at stage E14.5 with that of *Ror2*$^{-/-}$ humerus at the stages E14.5. and E16.5. We found 100 differentially regulated genes, which we started to characterize by automated in situ hybridizations on sections. Many of the examined genes are expressed in cartilaginous elements in the same or in adjacent regions as the *Ror2* expression. Our findings suggest that we were able to identify interesting genes involved in bone formation and possible downstream target genes of the receptor tyrosine kinase *Ror2*.

P1143. Expression analysis of Ufd1l, a gene deleted in DiGeorge syndrome, during mouse embryogenesis

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Ufd1l is the murine homologue of UFD1L, that is deleted in DiGeorge syndrome (DGS; OMIM 188400). Ufd1l-/- mice die before organogenesis, suggesting that the activity of this gene is crucial for embryonic development. In order to understand the biological role of Ufd1l during development, we investigate its expression during mouse embryogenesis. We collected RNA and protein from total embryos at different developmental stages from 4.5 to 18.5 dpc. RNA analysis was performed by quantitative real-time PCR (QRT-CR) using the ABI7000 apparatus in a multiplex PCR assay using

the rRNA18S as a control gene. Protein analysis was performed by Western blotting on protein extracts using a monoclonal antibody obtained after immunization of rabbit with synthetic oligo-peptides designed on the N-terminus sequence of the protein.
QRT-PCR assay demonstrates that Ufd1l mRNA is expressed during all the developmental stages investigated, with a continuous increase from stage 4.5 to stage 18.5. A Pearson correlation analysis demonstrates a positive correlation between development and the increase of Ufd1l expression (p= 0,016). RNA expression pattern was confirmed by protein analysis; in fact Ufd1l protein is present from 4,5 to 18,5 and its amount increase during development. We demonstrate that disease-target organs like heart, show the same pattern of expression, with a high level around stage 18.5 dpc.
These results demonstrate that Ufd1l is a dosage-sensitive gene and that its expression is strictly dependent from the developmental stage, so a perturbation and/or misregulation of this gene during development might contribute to the phenotypic spectrum of DiGeorge syndrome.

P1144. *FACL4* and mental retardation: protein characterization and cellular knock-out model

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In 2002 we identified *FACL4* (fatty acid-CoA ligase 4) (or *ACSL4*) as a new gene responsible for non-syndromic X-linked mental retardation (MRX). The gene encodes for a protein which adds Coenzyme-A to long chain fatty acids, with high preference for arachidonic acid. It is expressed in all tissues except for liver and presents a brain-specific isoform resulting from alternative splicing and containing 41 additional N-terminal idrofobic aminoacids. In order to establish how a mutation in this protein causes mental retardation we have characterized FACL4 expression in normal neuronal and non-neuronal cells. Western blot analysis on protein isolated from brain, lymphoblasts and the neuronal cell line SH-SY5Y demonstrated that FACL4 presents two different forms in lymphoblasts. On the contrary, in brain and neuronal cell lines there is a third form with higher molecular weight, presumably corresponding to the brain-specific isoform. Immunofluorescence experiments in the neuronal cell line with markers for different cell compartments demonstrated that the protein is located in the medial golgi. Analysis of membrane lipid extracts (total and from rafts) failed to demonstrate different lipid composition (in cholesterol and ceramide) between lymphoblasts of patients and controls. In order to define the possible role of FACL4 protein in neurons we have analyzed the consequences of FACL4 absence on neuronal differentiation in SH-SY5Y cells by employing antisense technology. This analysis has shown that differentiating cells lacking FACL4 expression present significantly longer cell processes with respect to cells expressing the protein, suggesting a role for FACL4 in membrane synthesis or recycling processes.

P1145. H-prune overexpression in transgenic epidermis induces a skin inflammation phenotype: evidence for a role in psoriasis.

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Inflammatory skin disorders, including psoriasis, represent an emerging field of study, although few new therapeutic agents have been described recently. Here, we have created an animal model with epidermis overexpression of h-prune, a newly identified cAMP phosphodiesterase. This mouse model exhibited hyperproliferation and an inflammatory skin phenotype, demonstrated by the altered expression of keratin 6, keratin 14 and cyclooxygenase-2. Cytokines, including interleukin-1β, interleukin-6, RANTES, interleukin-20 and its receptors, were also found to be overexpressed, thus showing the induction of inflammatory processes linked to psoriasis. Moreover, in human psoriasis patients, we show an altered pattern of h-prune expression in twenty out of thirty-nine skin cohorts, evidence of a preferential high concentration in the spinous and granular skin layers. Using dipyridamole, a selective h-prune phosphodiesterase inhibitor, we observed a reduced h-prune activity and inflammatory process in mice skin. These results suggest that h-prune overexpression in the epidermis is a contributing factor to psoriasis, and thus that therapeutic agents that specifically inhibit its activity may prove useful in the treatment of inflammatory skin disorders.

P1146. Heteroduplex detection with the CEL endonuclease-based (CEL1) "Surveyor mutation detection gel kit" for standard gel electrophoresis.

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Background. CEL1 is a plant endonuclease, able to cleave DNA with high specificity at sites of base-substitution mismatch and DNA distortion. CEL1 is celery-derived and cuts both strands of heteroduplex DNA on the 3' side of any mismatch site. CEL1 has been proven to recognize both insertions/deletions and single base substitutions in human and animal genes at a rate of efficiency that varies with the sequence of the mismatch.
Methods. We tested the sensitivity of CEL1 (Trangenomic-Omaha,NE) in 20 amplicons carrying known mutations identified by prior sequencing from 13 exons of 8 different genes (SCN5A, Cx26, TAZ G4.5, KCNQ1, TNNT2, HFE, FBN1, MYBPC3). 13 wildtype(WT) amplicons were used as negative controls. We also tested CEL1 specificity, in terms of identification of the type of gene defects (deletions, insertions and single base substitutions). Amplicons were obtained by proofreading-Taq based PCR. The PCR conditions were maintained as in routine. Heteroduplexes were generated by a standard thermalcycler program: 95°C10'; 95°C-85°C(-2°/sec); 85°C-25°C (-0.1°/sec); 4°C. CEL1 digestion was performed and controlled according to the manufacture protocol on 200ng of heteroduplex DNA. Electrophoresis was run on 3%nusieve,1%agarose gel.
Results. CEL1 detected 18 of the 20 heteroduplex mutations (sensitivity 90%). Among the 18 heteroduplex, CEL1 identified 3/3 deletions, 2/2 insertions and 13/15 single base substitutions. Results were confirmed in 4 sets of repeated experiments. WT samples didn't generate false positive results.
Conclusions. CEL1 is a fast, low cost, sensitive and reproducible tool for detection of heteroduplexes, both known and unknown, and independently on restriction sites.

P1147. Transcriptional control of GLI3 gene expression

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Limb defects present an excellent model for the study of signaling pathways. Understanding these pathways allows not only to uncover the complex mechanism of limb patterning, but to elucidate at the same time basic molecular tools governing development of the human body. Loss of the proper control at crucial steps of the signaling pathways results in tumorigenesis. Three paralogous members of the GLI gene family, GLI1, GLI2, GLI3, translate signals of the Sonic Hedgehog protein into specific patterns of gene expression. Their co-ordinated function appears to determine a GLI-code which, in the limb, directs pattern formation in anterior-posterior direction. In humans, mutations affecting the GLI3 gene cause polysyndactyly syndromes such as PHS, GCPS, PAP-A/B and PPD-IV. Whereas our understanding of functional domains of GLI proteins emerges from analyzing the impact of specific mutations within GLI3, factors controlling the localized and timely expression of GLI genes during development are unknown.
To identify cis-regulatory elements controlling expression of GLI3, sequences upstream of the transcription initiation site were analyzed by transfection assays and mutagenesis for their capacity to function

as promoter. Intron 1 of the GLI3 gene contains an extended region of very high sequence homology between human and mouse DNA which might contain potential cis-regulatory elements. Expression constructs using the identified endogenous GLI3 promoter supplemented by fragments of intron 1 were generated. Transfection into suitable cultured cells followed by dual luciferase assays identified sequences which regulate the expression of a reporter gene.

P1148. Gene expression profiling of patients with proteinuric diseases

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Proteinuria, the loss of proteins in urine, is a hallmark of kidney glomerulus dysfunction. Recent studies of congenital syndrome of finnish type (CNF) and identification of NPHS1 gene responsible for the disease have shed more light to the field. However the exact mecanisms which lead to glomerular damage and proteinuria are still poorly understood. Our final goal in this project was to develop gene expression microarray which might serve as a diagnostic/prognostic tool for the proteinuric diseases and to test it. For candidate genes selection we performed several microarray experiments with kidney RNA from NPHS1 knock-out mice and CNF patients using large scale arrays. Differentially expressed genes from these studies as well as literature mining were subjects for the final choice of genes. To test the new array three groups of patients (focal segmental glomerulusclerosis, IgA nephropathy, diabetic nephropathy) and control individuals were screened. Differentially expressed genes for all groups compared to the controls were identified using TTest and Significance Analysis of Microarrays (SAM). A two dimensional hierarchical clustering was performed to test the array's ability to distinguish between different disease groups. The results show that the array holds a potential to be used as diagnostic/prognostic tool for the proteinuric diseases.

P1149. INGENOtyping - high speed generation of rodent animal models for gene function analysis

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Mouse and rats are the model of choice for *in vivo* studies of gene function but the development of allelic variant is time consuming and costly. In the past, generation of mouse models was dominated by gene targeting through homologous recombination, which requires sophisticated ES cell manipulation and chimera production. In rats such a technique is even not possible due to the lack of suitable ES cells. To fully exploit the power of genetics a collection of animal models displaying each a different allele of the same gene would be ideal. Here we report on INGENOtyping, an ENU-based approach allowing the several fold faster production of allelic variant animal models. INGENOtyping is based on Ingenium's mutant mouse or rat G1DNA archives which are compliled with a corresponding frozen sperm archive or derived fom living cohorts. As the mutagen ENU acts on the level of spermatogenesis, each G1 offspring is heterozygous for a unique set of point mutations including null, hypomorphic and hypermorphic alleles. PCR coupled with heteroduplex analysis allows a rapid screen of the DNA archive for mutant alleles for any given target gene. Identified models representing alleles most suitable for research are recovered either by *in vitro* fertilization or by matings. Thus, homozygous mutant animals are available for phenotypic analysis in approximately four months. We will present examples with data from our INGENOtyping screen of mutant genes involved in signal transduction and the onset of obesity.

P1150. Segmental duplications in human, mouse and rat genomes, and regions of break of synteny

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We have tested the hypothesis of a potential role of segmental duplications in breaks of synteny (BOS) between chromosomes of rat and mouse, which are evolutionary close organisms, whose full genomic sequences are available. AxtNet alignments between mouse and rat genomic sequences where obtained from the UCSC. Over 1.5 million anchors were chained into 1953 syntenic segments spanning more than 250 kb in the mouse sequence. In turn, those were further grouped into higher order structures and lead to total of 102 synteny blocks that covered 85% and 87% of the mouse and rat genomes respectively. These synteny blocks defined 82 and 81 regions of BOS, respectively in mouse and rat genomes. We examined the GC content, repeat composition and presence of segmental duplications in a region of +/- 25 kb around the boundaries of BOS in both species. Although slight differences in GC composition and in repeat content were observed, they were not significant. Segmental duplications where found in 12% and in 8% of the BOS boundaries in the mouse and rat genomes, respectively. Although for some chromosomes the presence of segmental duplications was significantly higher than expected in a random distribution of BOS, it was not the case for the global genomes. Lack of association between BOS and segmental duplications in mouse/rat comparisons, could be due to low quality in the assembly of regions containing segmental duplications and lack of statistical power to detect an association due to the small number of BOS between these organisms.

P1151. Analysis of dopamine D2 receptor 3'UTR for functional sites and polymorphisms

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The dopamine D2 receptor is a major target for therapeutical drugs in psychiatric disorders. The 3'UTR of the D2 receptor gene (DRD2) encompasses 1119 bp. We sequenced the complete 3'UTR of 21 subjects, confirmed two frequent and one rare SNP named R22316, S22640, and K22989, respectively and identified a new rare SNP (Y22521). Based upon the sequences of non-human primates the region flanking R22316 is highly conserved, whereas S22640 is followed by a dinucleotide deletion in humans. Two independent studies reported an association of genotypes of R22316 and quantitative traits related to the outcome of withdrawal treatment in alcoholics (Pharmacogenetics 2001, 11:647-53 and 1997, 7: 271-81). Compared to genotypes of other SNPs located in the promoter region, introns, coding regions, and further downstream, the association with R22316 was the strongest one. BLAST search revealed short sequence stretches identical to 17-18 nucleotides flanking R22316 in 3'UTRs of at least two other genes expressed in the human brain. Therefore, we hypothesized functional elements, i.e. protein binding sites, located in the 3'UTR of DRD2 mRNA. Using protein lysates of a human neuroblastoma cell line we analyzed radiolabeled allelic DRD2 3'UTR transcripts of various size by UV crosslinking assay. Following RNase treatment the crosslink products were separated by electrophoresis and analyzed by autoradiography. The results suggest quantitative differences in protein binding of the allelic variants of R22316. In order to confirm these findings, RNA constructs similar in size to the conserved elements will be analyzed.

P1152. Sequence database resources at the EBI

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The EMBL nucleotide database <www.ebi.ac.uk/embl> continues to play its role in the International Sequence Database Collaboration (INSD), by accepting, storing and distributing nucleotide sequence data. We strive to provide genome centres with simple data pipelines to submit data, and also to have the best environments for

submissions by individuals. In addition to accepting primary sequence data, we now accept Third Party Annotation (TPA) entries. These are entries where users have provided re-annotations/re-assemblies of sequences already present in DDBJ/EMBL/GenBank and owned by other groups. In addition to sequence entries, we also run a database of alignments, EMBL-Align <www.ebi.ac.uk/embl/Submission/alignment.html>.
The nucleotide database also provides much of the primary protein sequence found in UniProt. UniProt has been formed by uniting the Swiss-Prot, TrEMBL and PIR protein database activities <www.UniProt.org> in order to provide a single, centralized, authoritative resource. The UniProt databases consist of three database layers:
(i) The UniProt Knowledgebase (UniProt) provides the central database of protein sequences with accurate, consistent and rich sequence and functional annotation.
(ii) The UniProt Archive (UniParc) provides a stable, comprehensive, non-redundant sequence collection.
(iii) The UniProt NREF databases (UniRef) provide non-redundant data collections based on the UniProt knowledgebase in order to obtain complete coverage of sequence space at several resolutions.
Characterisation of functional protein domains helps provide vital clues in deducing protein function. We provide a comprehensive, high-quality database of protein domains at InterPro <www.ebi.ac.uk/interpro> through the integration of data from nine member databases.

P1153. Patentability Options within Bioinformatics

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This study examines the current European legal framework for the protection of bioinformatic inventions. Firstly, the work describes the expansion of bioinformatic inventions business and its economic relevance. It shows the recent European legal status regarding: (i) patenting of computer programs, databases and algorithms; (ii) copyright protection of databases. In addition to the patents for gene sequences, many patent applications have been made in Europe which claim different aspects of bioinformatics tools. Bioinformatic inventions that provide tangible results or have a practical application are not excluded from patentability. European Patent Office (EPO) decisions such as Vicom (Case number T 0208/84) are here analysed. The work explores some strategies towards university bioinformatic inventions patenting. Useful database “free access” options - when no legal use restriction is imposed specially to academic institutions - are also shown.
The research method adopted consisted in interviews with patent agents. Secondary sources of information such as regulations, EPO decisions, documents reports and press reports were also useful.

P1154. Shh-dependent regulation of proliferation, differentiation and apoptosis of myogenic precursor cells

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Muscle satellite cells define a unique lineage of myogenic precursor cells that arise late in development and are believed to form a stable, self-renewing pool of stem cells in adult muscle where they function in tissue growth and repair. It is also assumed that a regulatory disruption of growth and differentiation of these cells results in tumor formation. Here we show that the division of murine myogenic precursor cells is regulated by Sonic hedgehog signalling. In addition, Sonic Hedgehog (Shh) treatment of both C2C12 and satellite cells prevents their differentiation into multinucleated myotubes and inhibits apoptosis initiated by serum deprivation. The effects of Shh were reversed by simultaneous treatment of the cells with cyclopamine, a specific inhibitor of the Shh signalling. In summary, these results suggest a crucial role of Shh signalling in the formation and maintenance of muscle satellite cells and may shed light on the mechanisms of myogenic tumorigenesis.

P1155. Prediction of candidate disease genes and regions by automatic integration of positional, functional and sequence data.

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Data on functional aspects of genes, gene products and genomes, and relationships with human and animal biology and diseases are increasing exponentially. As a result, scientists will use an ever increasing time to keep up with relevant information, and to link available data into a proper biological context, including relevance for human disease. One solution is to develop automatic systems that can integrate, evaluate and filter all data resources, ideally to dispose of the vast excess of irrelevant data, and to present the relevant data in a condensed, easy-to-read, yet flexible format. We have used Mendelian Cytogenetics Network database (MCNdb)(http://www.mcndb.org), containing >2700 disease-associated balanced chromosomal rearrangements (BDCRs), associated with >6000 chromosomal breakpoints and >8000 trait descriptions, as an ideal starting point for prototyping an automated association system. Each trait in an MCNdb case is queried against OMIM and PubMed, to create a list of textually associated chromosomal positions. Also, OMIM and PubMed are queried by the positional data from the involved breakpoint regions, creating another list of disorders and associated traits. These two lists are then compared. Positive hits are presented at various levels, ranging from candidate chromosomal regions to specific candidate genes. Screening of chromosomal rearrangements in MCNdb associated with a known disease gene correctly identified most known disease genes as a candidate for the traits involved. Further work will include more types of symbols and their relations, i.e. gene homology calculations, and tuning of inter-relationel weights of the system by using known disease genes as training sets.

P1156. Integrative analysis and interpretation of gene expression data

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The BioXM™ Master Suite is a framework that supports integrative analysis of high-throughput biological data. The system is composed of a universal data management platform, which organizes data within projects, and data analysis tools, which provide methods for performing calculations. The BioXM workflow allows results from one method to be used as the input for another method. The tool also provides annotation tools for organizing data which facilitates analysis interpretation. For example, controlled vocabularies, such as catalogs, ontologies or thesauri, can be used for systematic annotation of automatic integrative analysis.
The BioXM Gene Expression Analysis Tool is a framework for organization, analysis and interpretation of gene expression data. Data analysis includes gene clustering according expression profiles (e.g., dose or time-response curves) and hierarchical grouping of experiments according to expression patterns. The software provides detailed information, such as gene name, EC number, functional classification and protein descriptions, for each gene in a cluster. The system allows clusters to be analyzed with respect to functional classification and indicates overlapping categories and functional relationships. Analysis and evaluation of metabolic pathways based on gene expression data are also provided to facilitate interpretation of gene expression data.
Relevant knowledge located in public or proprietary databanks can be integrated and searched using the BioRS™ Integration and Retrieval System.

P1157. Biochemical human genetics and Inborn Errors of Metabolism (BHG & IEM): 15 years experience of teaching

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There is special structure of medical education to improve professional skill in Russia. It provides for periodical training at the

institutes of advanced medical studies like St. Petersburg Medical Academy for Postgraduate Education (SPbMAPE). Different training courses prolong 1,5-2 mo and include substantial number of the questions of the biochemical human genetics (BHG) and inborn errors of metabolism (IEM) that are the most difficult part of Medical Genetics for our physicians. During 15 yr 120 courses have been organized by the staff of the Department of Medical Genetics (SPbMAPO) in St.Petersburg and all over Russia. About 4000 physicians of the basic clinical specialties have been studied. One has to ascertain the standard of genetic knowledge on BHG and IEM is tragically low: correct answers on our test questions make up 40-50 per cent. Blitz-questioning on this subject showed our respondents to have enumerated 89 terms but only 14,6 % of terms were really related to BHG and IEM (for example, 28%of doctors wrote a-fetoprotein, Rh -56%, ABO blood group-35%, PKU-24%, "PCR"-22%), remaining words reflected the routine clinical biochemistry terms. At the beginning of BHG and IEM course most physicians express sense of horror and aversion to this subject of study. During this course the most recent information of metabolic basis of inherited diseases is given to our participants using well-documented cases of metabolic disorder from AJHG, EJHG, Electronic-Database Information etc. Dissatisfaction of our physicians concerning real possibility to diagnose IEM is the main result of this course.

P1158. High frequency of deafness in a genetic isolated population due to Founder effect

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Deafness is the most frequent sensorineural defect in human. More than 50 % of deafness is due to Genetic background. Inheritance of deafness has locus heterogeneity and it inherited as, autosomal dominant, autosomal recessive, x-linked recessive and mithochondrial. It also has incomplete penetrance and variable expression. Villages of HAJIABAD, AGHAGONI and GHALE NOO (South East of Tehran, Pakdasht city) have more frequency of deaf people compared to country frequency (1.3%). To determine the reasons of high frequency of deafness in these are as, notify to high risk individuals and prevention of deaf child, this research was done. All deaf people and family were counselled, their pedigree were drown. Results showed that mentioned villages are originally same and high frequency is because of founder effect. About 500 years ago, some people emigrated from Khoramabad city (Center of Iran) to these areas which some of them were carrier for deafness gene. Intratribal marriages led to increase of frequency of this gene. Based on Hardy-Weinberg law, approximately 34% of these isolated populations are carriers. Deafness in this population is non-syndromic and autosomal recessive with variable expression. It is noted, that Mellitus diabetes and mental retardation have high frequency in addition to deafness. Up to now similar isolated population with high frequency of deafness has not been reported in the world.

P1159. National External Quality Assessment (EQA) in Cytogenetics using Validated slides and Web-based approaches.

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Current limitations of Cytogenetic EQA Schemes include providing laboratories with comparable cases, and opportunity to make additional investigations.
The introduction of validated slides has been successfully used for Postnatal EQA in the UK. Validated slides were obtained from several accredited labs. The results and lessons learnt after two rounds of EQA with validated slides will be discussed.
UKNEQAS in collaboration with Waypoint Systems are piloting a web-based approach that closely models a diagnostic laboratory's case handling. The user chooses which cells they wish to analyse from a series of metaphases, and then can select any additional investigations e.g. C-banding, FISH, or requesting parental bloods. Upon completion of the case they submit the karyotype and report for assessment. The program provides full audit trail information.
This web-based approach for performing EQA will be piloted in this year for both FISH (Haematological and Constitutional cases) and prenatal rounds.
This web site has been developed with an additional training application. Trainees can log on and re-analyse any archived EQA case. Trainees select the metaphases and choose additional tests to interpret and report the case. The trainee (or trainer) can then visualise the route taken through the web page and compare it with an optimal route. This optimal route can follow the laboratory's EQA submission or be input by the trainer to exclude those modules not completed by the trainee e.g. FISH.
This project was funded by CPA UK Ltd. and will be discussed in more detail with specific application to a laboratory setting.

P1160. Possible perhaps new Approach to preconceptional and while conceptional prophylaxis of foetus deflections

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In 236 families lymphocyte transfusion was carried out in early period of pregnancy. Among them there are families with one miscarriage in the anamnesis - Pregnancy Loss (PL) made up 85 families; with 2 miscarriages - Usual Pregnancy Loss (UPL) - 106 families and families with 3 and more miscarriages (UPL 1) made up 46 patients. In 22 % of cases one group and Rhesus compatible father's lymphocytes were used, in 78% of pregnancies were used compatible lymphocytes of third persons. The percent of successfully ended pregnancies after applying lymphocyte transfusion in complex treatment in early periods of pregnancy constituted at PL- 97,6%, at UPL - 89,6%, at UPL 1 - 86,7% and the total per cent of all successfully ended pregnancies made up 92%, that corresponds to literature data. To our mind the effectiveness of connected with immunitycorriginal property of donors' lymphocytes.
Taking into consideration that the persistering infections takes place in 64 cases among women with PL and especially with UPL, this circumstance increases probability of foetus infectioning, and inneruterine infection in 5% is cause of inborn anomalies (K.Nelson,1987). Based on nonspecifity of immunity lymphocyte transfusion one can assume that it leads to the forming of valuable immunity respond on persistering infection agents that finally should decrease foetus complications mentioned above. According to recommendations introduction of donors'lymphocytes before pregnancy period and during it and taking into consideration nonspecific immunity correction, this method can be referred to the method of preconceptional and while conceptonal prophylaxis of foetus deflections.

P1161. Level of education in families with Pregnancy Loss taking medical advice in Medico-Genetic Centre

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For determining the level of education was analyzed the selection of families addressing to Medico-Genetic Centre on the occasion of Pregnancy Loss at the account since 1985 till 1998consisted of 1374 patients, women - 700; men - 674.
Comparative data of education level of selection and population

Level of Education	Medico-Genetic Centre % n=1374 p.	Population % Goskomstat RF,1995 n=105688 1000 people
1.Higher Education	28,3115	12,05435
2.Incomplete Higher	2,838428	1,82613
3.Secondary special	41,41194	20,54538
4.Secondary general	25,18195	29,31837
5.Incomplete Secondary	2,183406	22,46613
6.Primary Education	0,07278	13,78964

Data obtained is evidence that 30% of the total number of people with incomplete secondary and primary education do not apply to Medico-Genetic Centre on the occasion of reproductive losses due a number of reasons.

It is shown, that the educational level of addressing women statistically doesn‘t considerably differ from the educational level of men.
So this data is evidence of the fact that minimum 30% of population didn‘t use available Medico-genetic assistance.
This circumstance sets the tasks for the above mentioned Centre to seek new approaches for spreading special knowledge among social groups with low level of education.

P1162. Karyotyping on the web

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Almost all human cytogenetic studies involve the examination of dividing blood cell population by blocking cell division at metaphase with subsequent processing and chromosome staining by banding techniques. Karyotypes are prepared by cutting up a photograph of metaphase chromosomes, matching up homologous chromosomes and sticking them back down on a card - or nowadays by getting an image analysis computer to do the job. Teaching a student how to detect and interpret even the most common chromosome abnormalities is a challenge in a developing country where the laboratorial facilities are not always available. Therefore, in this work we present an educational software for teaching undergraduate students of Medical and Life Sciences Courses how to arrange chromosomes in the form of karyotype. The user, using drag-and-drop, is dared to match up homologous chromosome. For that, we have developed a free full access web site (http://www.biomol.net/cariotipo/) for hosting the software. This web site also offers a theoretical introductory section with basic concepts about karyotype. Up to now the software has been successfully applied to undergraduate courses at the University of Rio de Janeiro (UNIRIO). The students have approved the software; to them the similarities with the well-known game solitaire turns the exercise more exciting and provides additional stimulus to learn and understand karyotype. Professors have also used the software as complementary material in their regular classes. Based on their testimonies the software was a helpful tool for explaining the karyotype assembly in Portuguese language.
Financial support: FAPESP, UNIRIO.

P1163. Opinions on genetic testing and self-reported skills among general practitioners, gynaecologists and paediatricians

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Objective To investigate opinions of general practitioners (GPs), paediatricians (PEDs) and gynaecologists (GYNs) on genetic testing and to explore their self reported skills.
Methods A questionnaire developed by Hofman et al (Acad Med 1993; 68: 625-32) containing questions about genetic testing and related skills, was adapted to the Dutch health care system. Questionnaires were sent to randomly selected GPs (n=200), GYNs (n=300) and PEDs (n=265).
Results The response rate of GPs, GYNs and PEDs was 64%, 69% and 72%, respectively. In total, 22% of GPs, 30% of GYNs and 36% of PEDs were (very) likely to routinely offer a predictive test for treatable common disorders even when no others would do so in their specialty. Once common practice 75%, 91% and 89%, respectively would do so.
Of the GPs, GYNs and PEDs, 34%, 39% and 47%, respectively answered that they thought it was appropriate to use prenatal testing by parents for sickle cell anaemia. Almost all physicians thought that this was appropriate for Duchenne muscular dystrophy and for Cystic Fibrosis, whereas only 26% of GPs, 28% of GYNs and 17% of PEDs thought that this was appropriate in the case of hereditary breast cancer.
The majority of physicians felt (very) confident in handling all kinds of test-related situations (67 - 92%).
Conclusion Reservations were present among these physicians for routinely offering predictive testing. The opinions on prenatal testing varied widely for different diseases. Most physicians felt their skills were adequate.

P1164. Evolution of an external quality assessment for Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease type 1 (CMT1) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. CMT 1A is the most frequent autosomal dominantly inherited form caused by a 1.4-Mb tandem duplication in chromosome 17p11.2-12 comprising the peripheral myelin protein 22 (PMP22) gene. Genetic testing for CMT1A is routinely offered in diagnostic molecular genetics laboratories and performed using a wide variety of methods such as detection of CMT1A junction fragments, STR and RFLP analysis to assess dosage or the presence of 3 alleles, FISH, quantitative PCR to detect PMP22 dosage and other techniques. In 1999, a pilot External Quality Assessment (EQA) scheme for CMT was offered by the European Molecular Genetics Quality Network (EMQN). From 14 registered laboratories out of 12 European countries a total of 11 participated. In 2000 20 laboratories participated. One genotyping error leading to a msidiagnosis was detected in the 2000 EQA. Thirty laboratories from 15 countries reported for the 2001 CMT EQA. Although two genotyping errors occurred, these errors did not lead to misdiagnosis of the CMT1A duplication, due to the use of multiple methods. In 2002 no genotyping error occured, but in 2003 with 39 participating laboratories one genotyping error was registered. The obvious tendency, a higher number of participating laboratories resulting in more genotyping errors, is an indicator for the necessity of EQA schemes. The improvement of existing European guidelines for genetic diagnosis of CMT (www.emqn.org) is necessary to harmonise laboratory standards for the genetic diagnosis of CMT.

P1165. Validation of a decisional instrument for preconceptional carrier screening for cystic fibrosis and/or haemoglobinopathies

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Objectives: Cystic fibrosis (CF) and haemoglobinopathies (HbPs) are autosomal recessive disorders. Preconceptional carrier screening allows couples at risk to arrive at informed reproductive decisions. Couples are eligible for CF- and/or HbP-screening based on their ethnicity. Screening all participants for both disorders is inefficient and costly. However, selecting people on the basis of their ethnicity may lead to stigmatisation and discrimination. Therefore, two decisional instruments were developed to support couples in making the right decision about which disorder should be screened for (CF, HbP, both disorders or none). The validity of these decisional instruments was examined.
Methods: The instruments, both combining questions about the ancestors’ origin of both partners, were: (A) a flow chart and (B) a questionnaire with pictures of the original geographical areas at risk for CF and HbP. People (n=112) of different ethnic background, used the instruments and arrived at a decision about the disorder(s) to be screened for. Subsequently, they were interviewed about their origin to validate their decision.
Results: Instrument A and B led to the right decision in 88% (99/112) versus 91% (102/112). Instrument B was preferred by the majority of the participants (57%). In 12/17 cases with an incorrect decision, it resulted in a screening proposal for less disorders than indicated. Of the participants 34%, 31%, 32% and 3% was eligible for CF-screening, HbP-screening, for both and for neither CF- nor HbP-screening, respectively.
Conclusion: Both decisional instruments were valid. Instrument B will be used in a preconceptional carrier screening study for CF and/or HbP.

P1166. Influence of genetic testing and information on the people with trisomy 21

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In Split and Dalmatian county area 170 children with syndrome Down are registered in this parents organistaion and The parents organization *Split-21* define their project: to assure better socialisation, education of parents, creating a new athmosphere in the public, breaking barrieres that are created by prejudice.
All aspects of illness or condition must be explained: causes, development, prognosis, other difficulties, possibilities of inclusion in society (with a positivistic approach). Follow up must be preceded multidisciplinary. In the program they included psychological, defectological and logopedic treatment of DS patients, prenatal diagnosis and genetic testing too. For the children they seeks: right to have their own way of living, adequate education, to be stimulated and encouraged, to be employed!, to be included as equals in society, to express their own opinion, to selfdetermination, to have social security and benefits, to be informed about genetic testing, to be involved in genetic counseling, to be asked about transplantation of organs with explanation suitable for their mental retardation.*
Bringing up a disabled child is difficult and can make you feel like a martyr if you let it, but it can also bring out the very best in both yourself and the people around you* (a father of a DS patient).

P1167. A pilot study of psychosocial aspects of genetic testing for hereditary cancer predispositions in Czech Republic.

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Studies focused on psychosocial factors associated with genetic tests for predisposition to various types of cancer revealed important findings about patients' attitude towards testing and prevention, motivation, psychological support needs, concerns about discrimination and communication of the genetic test results within the family. Nevertheless, it can be expected that these factors would vary in different countries due to the social and cultural background. The study aim was to explore these factors in patients undergoing the testing for hereditary cancer predisposition and to identify the differences and needs which may be characteristic for patients in Czech Republic.
Sixty-eight questionnaires containing 20 multiple choice questions were distributed to patients who had received pre- and post-test genetic counselling. From 52 patients who completed the questionnaire 27 were tested for predisposition to HBOC, 11 to HNPCC, 11 to FAP and 3 to LFS.
Comparison with previously published studies indicated the difference in the principal motivation for undergoing the testing ("to learn about children's risk of cancer"), disclosure of test result to the relatives (all of the patients informed at least one member of the family, 71% informed all first degree relatives, 19% informed distant relatives) and lower concerns about discrimination (10% - 14%). The attitudes towards testing, prevention and psychological support needs were similar to other studies.
There are several differences which might be characteristic for Czech patients. Due to sample limitation future studies also specific for each cancer predisposition should be performed to explore further these initial findings.

P1168. The Internet - An appropriate source of information for patients with genetic disorders?

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As the Internet is becoming more and more popular the number of people seeking information on health problems is growing. According to clinical experience and a study performed by our department the Internet is increasingly becoming a resource for counselees seen in our genetic clinic.
We wanted to assess the usefulness of the net and interviewed 152 individuals. Results show that in 2002 about every third counselee had used the Internet for personal information before genetic counselling.
The actual number of those who had been properly informed remained low, though.
One reason lies within the quality of the web sites, which are rarely reviewed by experts and may contain false information. Some information, though it may be pretty useful, is hidden amongst an abundance of websites that are listed under the same topic by search engines.
Most people are sceptical of trusting information on the Internet and are unaware of potential commercial interests.
We applied criteria, evaluated websites with genetic contents and assessed their reliability and accessibility. The criteria covered e.g. truthfulness, readability, design-features, identification and liability of their creators, and potential commercial interests.
English and German language websites for the most common genetic diseases and problems were assessed.
We aim to establish such criteria more broadly to help consumers of genetic related web sites to find and trust information relevant to their needs.

P1169. Monitoring of Congenital Developmental Defects in Nizhny Novgorod Region

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Systematic monitoring of congenital developmental defects in Nizhny Novgorod region has been taking place since 1999 by the cohort method. The method was applied on the population according to the basic principles of the international register.
Despite the short term, the database contains data on 110,000 newborn babies, of whom 2050 (1.86%) have congenital anomalies. The summary frequency of congenital developmental defects, which are to be registered, is 4.55: 1,000 newborn babies, which is in accordance with the average figures of the International Register. The frequency of particular nosologic groups is comparable with the data of EUROCAT, but the comparison of our own data with the results of the Federal monitoring of congenital developmental defects showed that the Nizhny Novgorod region belongs to the group of those subjects of the Federation where the highest frequency of defects of the neural tube is observed.
Analysis of the data from the region monitored also showed that the frequency of defects of the neural tube among the Nizhny Novgorod population, including prenatal cases, (0.88 per 1,000 newborn babies), is higher than in those countries of Europe (Hungary, France, England - 0.25-0.3 per 1,000) where preconceptional prophylaxis with folic acid is applied ($p<0.1$).

P1170. Birth Defects Surveillance and Prevention in Ukraine - Planning Implementation and Results

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A Birth Defect (BD) monitoring system based on international standards was introduced in Ukraine under the auspices of the United States Agency of International Development. Also, 5 Resource Centers (RC) were created to promote early diagnosis, treatment and prevention programs to sustain a BD Prevention National Alliance (BDPNA) for parents and care providers. An "International BD Information Systems" (IBIS) website was also developed.
The BD Monitoring System reports data to International bodies and RCs serve to enhance services, training, and postgraduate medical education programs as well as the needs of parental support groups and the BDPNA. IBIS became a popular resource (nearly15000 pages are read per week).
BD monitoring shows a prevalence of Neural Tube Defects of 2.1 per 1000, about 4 times as high as expected. Consequently, a national initiative to fortify flour with folic acid was initiated. Updated regulations and a pilot flour mill – bakery chain will permit imminent

pilot implementations to produce folic acid fortified bread.
BDPNA is a member of international organizations has attracted international partners. BD surveillance and care programs are now being expanded to include Early Interventions (EI). In Ukraine, EI resources are limited and are virtually centered in orphanages. Parents, BDPNA and RCs, along with other partners, created EI day care centers for infants and will provide EI for orphans. One aim is to decrease the likelihood that parents will need to relegate the care of infants to orphanages. Another aim is to prevent mental delays of biologically sound orphans through EI.

P1171. The Impact of a BD Web Information System (IBIS) on Birth Defects Programs

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A web-based International Birth Defects (BD) Information System (IBIS) was developed to enhance BD programs in Ukraine and Alabama. The design of IBIS takes into account that excellent information resources are available from the web, along with a plethora of mediocre and poor alternatives. The design also recognizes that the overwhelming majority of web resources are in English and that most are focused on specific anomalies. IBIS encourages partnerships, particularly the development of non-English fact sheets (FS).
IBIS is designed as a “navigation site” to facilitate access to information on BD, syndromes, genetics, teratology, parental support groups, as well as on scientific and academic issues. FS include the above domains in multi-lingual versions. When sources are lacking, IBIS partners develop original FS in versions for professionals and patients. A large pool of FS had to be developed in Ukrainian and justified a Ukrainian version of IBIS. A Spanish version of IBIS is in development.
The number of visitors to IBIS http://www.ibis-birthdefects.org suggests that the design and contents are valuable to a large number of international visitors (over 1000 daily). IBIS is a core resource for BD programs in Ukraine and in Alabama utilized to enhance clinical services and professional and public education. IBIS also is a resource for parental support groups and the Alliance for the Prevention of BD and accommodates IR developed by and for various parental support groups.

P1172. The Genetics File: getting the gene into general practice

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A practical genetics educational intervention was developed for GPs in Australia, following an earlier needs assessment responding to greater recognition of the role of genetics in primary care. The intervention includes a resource, available as a printed up-dateable folder, CD-ROM and online, and a highly interactive case-based workshop. The Genetics File resource contains sections: talking with families about genetics, familial cancers, haemochromatosis, adult-onset neurological conditions, testing during pregnancy, Down syndrome, fragile X syndrome, cystic fibrosis, newborn screening, glossary, and referral guidelines for familial cancers and clinical genetic services. Content includes information about the condition, which investigations should be performed, family implications, role of GPs in ordering tests and management, when and where to refer, including support groups, and information for families. Resource/workshop content was developed in close collaboration with GPs, specialists and consumers.
The educational intervention, attracting maximum CPD points, was rolled-out in July 2003 over 8 months, with a media and mailout campaign targeted to all GP practices in Victoria, and >20 workshops conducted. Its impact on GP practice of genetics is currently being evaluated. Evaluation includes a validated questionnaire measuring categories of knowledge, attitudes, skills and behaviour, administered prior to workshop, then 1 and 6 months later. Referrals to clinical genetics services, genetic testing and support groups are also included in the evaluation. Preliminary results from questionnaires indicate there is a significant improvement in all categories of responses, although it is still too early in the study to see whether this translates to changes in referral practice behaviour.

P1173. A pilot external quality assessment scheme for DNA sequencing

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Genetic testing is now a routine part of laboratory medicine. Many different technical approaches are used but DNA sequencing is widely regarded as the ‘gold standard’ for mutation detection. But how accurate is DNA sequencing, and can we be sure we are getting the correct result 100% of the time? External Quality Assessment (EQA), or Proficiency testing, is one approach to ‘independently’ quantifying the accuracy of the sequencing output from testing laboratories and sequencing facilities. Here we present the results of the second pilot EQA scheme for DNA sequencing run by the European Molecular Genetics Quality Network (EMQN) in 2003. The scheme aimed to test the ability of participants to sequence heterozygous, homozygous, deleted and normal DNA samples. Five samples were sent out to each participant; they were asked to report back to EMQN with their interpretation of any changes found and the raw data: sixty-four laboratories participated. The scheme design (including the associated problems) will be discussed and examples of results and errors will be presented.

P1174. Medical genetic service of Leningrad province: 2003 year.

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Medical genetics service is stationed at District Children Hospital. Its stuff includes a clinical geneticist and Laboratory of Cytogenetic study with laboratory personnel: a chief, 3 cytogeneticists, 2 laboratory-assistants and a laboratory technician. They work in close co-operation with the Department of Medical Genetics of the MAPE, Municipal Centre of Medical Genetics etc. Genetic service continue to realize neonatal screening for PKU and CH; selective screening for chromosomal pathology, second trimester prenatal screening for congenital defects (double-test); confirmation of hereditary diagnosis; medical care, long term inpatient and outpatient care, dietary management, genetic councelling. 98-99% of the children that were born in 2003 have been examined through neonatal screening. Two cases of PKU were diagnosed. Owing to realization of second trimester prenatal screening and genetic councelling for 126 persons we were able to detect prenatally syndromes Edwards (1), trisomy 21 (7), a case of inversion 9ph (1), mosaicism: 45,X/46,XY[2:15] (1); 46,XX/47,XXX[10:2] (1); 46,XX/47,XX+13[23:3] (1); 46,XX/47,XX+7[4:8] (1). Cytogenetic studies were performed for 488 persons. We have diagnosed trisomy 21 (3), trisomy 18 (2), Klinefelter syndrome: XXY (3); 46,XY/47,XXY (2); 46,XY/47,XY+X(p) (1); Turner syndrome: 45,X (3); 45,X/46,XX (2); 45,X/46,XX/47,XXX (1); a case of sex inversion: an inversion 9ph; male with 45,XY,t(21:13), male with 46,XY,inv11(p12q14); the children with mental retardation had marker chromosomes (3). We continue to create Register of inherited and congenital disorders among Leningrad province population

P1175. The External Quality Assessment in classical cytogenetics: the Italian experience.

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The Italian External Quality Assessment (EQA) in classical cytogenetics started on year 2000. EQA is the focus of two research Projects: the “National Project for Standardization and Quality assurance of genetic testing”(2000-2002) and the Project

”Genetics tests: from research to clinics”(2003-2004). Both projects are financially supported by the National Health System and co -ordinated by the National Institute of Health (Istituto Superiore di Sanità, ISS).
Cytogenetics Public Laboratories have been enrolled, covering all Italian regions and have been grouped in 6 inter-regional Working Units. The EQA scheme covers both prenatal and postnatal diagnosis, including cancer cytogenetics. Three trials have been performed untill now; participating laboratories numbered 35, 46, 49 in the I°, II° and III° trial respectively.
Our EQA has covered about one third of all Italian laboratories performing cytogenetics.
Laboratories sent to ISS Jpeg images relative to three metaphases together with two reconstructed karyotypes plus the written report. Images were selected according to a fixed schedule.
Evaluation took into account the analytical accuracy, data interpretation and reporting. All data were treated anonymously. A panel of experts reviewed quality of images and reports.
In the first two trials images had a good level of quality, while report formats were not homogenous and often missing important information. The evaluation of data relative to the third trial is in progress. An overview of all results will be illustrated and an effect of EQA in three years will be discussed.
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P1176. Views about the importance of genetics and professional confidence: Education to support the role of Cancer Nurse Specialists in familial cancers

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The West Midlands Family Cancer Strategy (WMFACS) ensures appropriate advice and treatment for people at risk of familial cancer in the West Midlands, UK. Cancer Nurse Specialists (CNSs) care for all cancer patients and work with the Strategy in cases of familial cancer. However, many have little genetics knowledge. A three day course for CNSs, “Assessment and Management of Familial Cancer”, was therefore established.
Using pre- and post-course questionnaires, participants rated the importance of genetics in their clinical practice and their confidence in their own genetics skills. Initially importance was rated high but confidence was low.
Course topics were generated by telephone interviews with participants and refined by specialists in cancer genetics and education. Topics included cancer genetic predisposition and inheritance; pedigrees; risk assessment; epidemiology; national surveillance programmes; ethics; patient perspectives; the genetic basis, surveillance, chemoprevention and surgical options for breast/ovarian and colorectal cancers; lab tests; and future WMFACS strategy. Delivery methods included interactive presentations, lectures, problem solving and practical sessions.
Participants’ confidence was higher after the course. On a scale of 1 (high) to 5 (low), participants rated: relevance to their educational and clinical needs: 1; and whether they would recommend this course to colleagues: 1 (median scores).
Education in genetics is essential for healthcare professionals (HCPs) working with specialist genetics services. Each HCP group can play an important role in developing courses tailored to their needs. Here we have demonstrated that appropriate genetics education can improve the confidence of CNSs in the genetic component of their role.

P1177. The Italian External Quality Assessment (EQA) in molecular genetic testing coordinated by the Istituto Superiore di Sanità: three years of experience

D. Taruscio, *N. Bruzzese, F. Censi, V. Falbo, G. Floridia, M. Salvatore, F. Tosto;*

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The Italian External Quality Assessment (EQA) in molecular genetic testing started on year 2000. EQA is the focus of two research Projects, both financially supported by the National Health System and coordinated by the National Institute of Health (Istituto Superiore di Sanità, ISS).
Public Laboratories have been enrolled, covering all Italian regions and have been grouped in 6 inter-regional Working Units. The EQA scheme covers four pathologies: Beta-Thalassemia, Cystic Fibrosis, Fragile-X and Adenomatous Polyposis of the Colon (APC gene). Three trials have been performed untill now (2001, 2002, 2003); participating laboratoriesnumbered 41, 50, 56 respectively.
DNA samples, obtained from peripheral blood or from lymphoblastoid cell lines using standard protocols, were collected through the Working Units co-ordinators and validated by the ISS.
Laboratories were invited to test, for each pathology, six validated samples. For each sample, laboratories were required to produce, within two months, raw data, interpretation of results and a final written report, using current methods and standard nomenclature. Results were collected by ISS for evaluation.
For each disease, a panel of experts reviewed results including raw data, reports and nomenclature. All data were treated anonymously, and at no time are laboratories identities revealed.
The overall data evaluation in three trials reveals a general high standard of analytical accuracy; on the other hand, written reports were not homogenous and often missing important information.
An overview of results and the effect of EQA in three years will be discussed.
Financially supported by the Project „Genetic tests: from research to clinics“-ISS

P1178. Early experience of a MultiDisciplinary Cardiogenetics Clinic in a Tertiary Referral Centre

B. Skaria, *H. Cox, A. Lucassen, J. M. Morgan, D. Wilson;*

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Background: Rapid advances in genetics has resulted in an increasing number of identifiable genetic conditions affecting the heart. Predictive and prognostic information can be obtained through the use of genetic tests both for individuals and their families.
Methods and Results: Wessex Cardiac Genetics Service serving a population of 2.9 million, was established in 2002.We have had 4 meetings in total, and we have discussed 24 index cases with the Long QT syndrome , Brugada syndrome , Familial Aortic dissection , Hypertrophic cardiomyopathy , Catecholaminergic Ventricular tachycardia , familial Ebstein’s anomaly , Arrhythmogenic Right ventricular cardiomyopathy , Duchenne muscular dystrophy , Pseudoxanthoma elasticum , Barth syndrome , Pompe’s disease , Marfan’s syndrome and familial Atrial Septal Defect with conduction defects .
From the index cases, we traced 234 family members, who have been offered counselling and genetic screening where appropriate. Management protocols have been developed for carrier females from Duchenne muscular dystrophy families in our region. Because of these meetings, we have been able to establish newer diagnostic mutation screening/tests for Long QT syndrome at our Regional Genetics Laboratory at Salisbury.
Conclusion: Establishment of multidisciplinary cardiogenetics clinics is a necessary component of Tertiary Referral Centres . It offers a ‘holistic’ management to patients and their families affected by various genetic conditions affecting the heart.

P1179. Family communication of BRCA1 and BRCA2 test results: Results from a telephone interview with 332 women tested within the German Consortium on Hereditary Breast and Ovarian Cancer (HBOC)

B. Schlegelberger[1], *D. Gadzicki*[1], *L. U. Wingen*[1], *I. Nippert*[2];

[1]*Hannover Medical School, Hannover, Germany,* [2]*University of Münster, Münster, Germany.*

To assess the Consortium‘s* approach for counselling and testing in HBOC families, 332 women who had obtained their test results at least six month earlier were interviewed by telephone (overall response rate: 74%) and asked to assess the congruity of statements from other women deciding to undergo testing with their own motives. There were significant differences between affected and unaffected

women regarding prediction of their own (62% versus 83%, $p<0.05\chi^2$) and their children's risk (84% versus 44%, $p<0.05\chi^2$). Another important motive was to make a contribution to cancer research (61%). The majority of women (86%) would opt for BRCA1/2 testing again, but significantly less mutation carriers than non-carriers would strongly recommend BRCA1/2 testing to others (55% versus 70%, $p<0.05\chi^2$). Motives still agreed upon in hindsight may be altered by women's overall experience of undergoing testing and by mutation status, and thus are of specific significance from the women's current perspective.
* Members of the Section „Human Genetics" of the German Consortium on Hereditary Breast and Ovarian Cancer:
Bartram CR, Beger C, Bosse K, Debatin I, Fischer B, Froster U, Gadzicki D, Goecke T, Grimm T, Grote W, Harder L, Horn D, Horst J, Kreuz F, Langenbeck U, Neumann T, Nippert I, Propping P, Schäfer D, Schlegelberger B (Spokeswoman), Schmidt D, Schröder J, Siebert R, Sperling K, Strenge S, Vogel W, Voigtländer T, Welling B.

P1180. Clinical genetics in Italy: strategy and organization

*A. Renieri[1], C. Pescucci[1], **R. Tenconi**[2];*

[1]Genetica Medica, Siena, Italy, [2]Genetica Clinica, Padova, Italy.

Clinical Genetics workshops are organized every 3 months. The aim is to offer support to the clinical diagnoses and education to young medical geneticists and pediatricians. Each workshop is organized in a full day format (from 10.30 a.m. to 17 p.m.) in a city of the middle of Italy (Bologna) to let people reach the city and come-back in the same day. The program includes a clinical genetics seminar, followed by the presentation of open clinical cases. A web site has been prepared, you can access to it by the Italian Society Human Genetics (SIGU) http://sigu.univr.it (and then click on Clinical Genetics) or directly through: http://www.unisi.it/ricerca/dottorationweb/genetica_medica/incontri_di_genetica_clinica/incontri.htm. All navigators will find the date of past and coming workshops, a list of reported cases, and a list of speakers. Only those who have a personal password can access to the full information of cases and to the slides presented during the seminars. The passwords are furnished to medical community upon a written request. Two week before the planned date of each workshop the case-examiner receives a clinical synopsis and photos of patient. One week before, all the material is available on the web site. After the workshop all comments (from the case-examiner and audience) are summarized in the web site. They represent an input for the follow-up the patient. A follow-up will be presented again after a due time. These workshops are accredited through the SIGU for Continued Education in Medicine (13 Credits for 2 workshops).

P1181. Decision-making on BRCA1 and BRCA2 testing: the participants' retrospective view

***D. Gadzicki**[1], L. U. Wingen[1], B. Schlegelberger[1], I. Nippert[2];*

[1]Hannover Medical School, Hannover, Germany, [2]University of Münster, Münster, Germany.

To assess the Consortium's* approach for counselling and testing in HBOC families, 332 women who had obtained their test results at least six month earlier were interviewed by telephone (overall response rate: 74%) and asked to assess the congruity of statements from other women deciding to undergo testing with their own motives. There were significant differences between affected and unaffected women regarding prediction of their own (62% versus 83%, $p<0.05\chi^2$) and their children's risk (84% versus 44%, $p<0.05\chi^2$). Another important motive was to make a contribution to cancer research (61%). The majority of women (86%) would opt for BRCA1/2 testing again, but significantly less mutation carriers than non-carriers would strongly recommend BRCA1/2 testing to others (55% versus 70%, $p<0.05\chi^2$). Motives still agreed upon in hindsight may be altered by women's overall experience of undergoing testing and by mutation status, and thus are of specific significance from the women's current perspective.
* Members of the Section „Human Genetics" of the German Consortium on Hereditary Breast and Ovarian Cancer:
Bartram CR, Beger C, Bosse K, Debatin I, Fischer B, Froster U, Gadzicki D, Goecke T, Grimm T, Grote W, Harder L, Horn D, Horst J, Kreuz F, Langenbeck U, Neumann T, Nippert I, Propping P, Schäfer D, Schlegelberger B (Spokeswoman), Schmidt D, Schröder J, Siebert R, Sperling K, Strenge S, Vogel W, Voigtländer T, Welling B.

P1182. The conception of a Dutch Centre for Society and Genomics

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The life sciences have entered a new and challenging era of research: the era of genomics. This will have a significant and as yet largely unpredictable impact on the way we see our world, our health and our environment. Some stress new possibilities of informed decision making for (prospective) parents and consumers („empowerment"), others fear for social pressure and exclusion. In order to assess and influence the societal impact of genomics, a Centre for Society and Genomics (CSG) will be established in the Netherlands. The CSG brings together a group of experts coming from various fields and backgrounds in a joint effort of building a common agenda for interdisciplinary research and societal communication. Notably, the CSG wants to further collaboration between empirical research (social science, communication science, epidemiology) on the one hand, and reflective research (philosophy, ethics) on the other. We believe that qualified research and reflection will help us to assess the impact genomics is bound to have in various fields (health care, food, environment). It is the Centre's objective to contribute to quality enhancement of the Dutch debate on Genomics.
The research agenda will include multifactorial health problems (such as obesity and alcoholism) as well as implementation issues (criteria for screening in the age of genomics, scenario study in the field of community genetics). In the domain of food, tailored treatment options will be studied as well as impact of genomics for the developing world. Communication and information will be studied in several fields. International collaboration is welcomed.

P1183. Impact of Resource Centers (RC) on Birth Defects (BD) Programs in Ukraine

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Six RCs were established as a component of BD Surveillance, care and prevention programs, as well as to activate the creation of parental support organizations, other community based resources and to expedite international partnerships.
The key elements of an RC are: trained information officers who are English-competent and knowledgeable of electronic information sources and web-technology; trained medical/clinical experts cognizant of BD, genetics and teratology; access to printed, electronic or web-based information resources; access to national and international consultants; RC staff located in major pediatric health care centers who, therefore, implicitly partake in RC activities; easy access to RC by professionals and the public through the extension of operating hours beyond standard working hours; electronic publication and dissemination of information resources developed by local authors; partnerships with medical and other teaching/training programs.
The impact of RCs is evident from the large number of visitors (>10,000 per year) and utilization of RCs by a variety of clinicians, allied health professionals, and parental grass root organizations, among others. RC teams published and disseminated novel information resources (e.g., growth standards for infants with or without BD and other disorders) and serve as sites for teleconsultations linking rural medical care providers with Ukrainian and international experts. We conclude that RCs represent an essential element needed for the introduction and sustenance of BD surveillance, care and prevention programs in Ukraine, as well as to provide stimulus for parents of children with BD to organize parental support groups.

P1184. Person-centered genetic counseling increases compliance to cancer screening programs

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Two to five percent of patients with colorectal cancer suffer from an inherited cancer syndrome known as hereditary nonpolyposis colorectal cancer (HNPCC). Persons at risk for HNPCC should undergo annual colonoscopy beginning at the age of 25. An interval of 3 years has been shown to be effective in preventing mortality and reducing late stage colon cancer. Genetic counseling of patients and persons at risk was carried out in a interdisciplinary setting (genetic counselor, clinician, psychologist) performed in a person-centered manner.
Patients and Methods: A standardized telephone interview was conducted with 81 persons 3 to 20 month after counseling. Statistical analyses were done with SPSS 11.0.
Results and discussion: Persons without colonoscopy were mainly persons at risk for HNPCC with a pedigree fulfilling the Amsterdam criteria (71%). 61 persons have had colonoscopy and were asked whether they had regular endoscopy before and after counseling. At time of the questionnaire 80.3% underwent screening examination regularily (interval $\leq$3 years), whereas before counselling only 43.9% of the persons requested for colonoscopy (p=0.004; Chi square 8.3). In addition, patients were asked to assess any inconvenience of colonoscopy and bowel cleansing (scale 1 to 10). Mean value for colonoscopy was 4.2 and 6,2 for bowel cleansing.
Conclusion: These data clearly indicate that in our cohort persons at risk with Amsterdam positive family history have a disposition to avoid cancer screening examinations. But person-centered genetic counseling significantly increased compliance to colonoscopy.

P1185. Quality evaluation of data interpretation and reporting

***E. Dequeker**[1], E. Girodon[2], M. Schwarz[3], M. Stuhrmann[4], I. Lubin[5], J. J. Cassiman[1];*

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Errors in any of the steps of a molecular genetic test may affect its results, interpretation, and use. Errors in tests that are time sensitive (i.e. prenatal) or are provided to asymptomatic persons (i.e. carrier testing) can pose significant problems.
Since 1996 European external quality assessment schemes (EQA) for cystic fibrosis (CF) have been organised. In 2003 (n=240), 33 US laboratories participated under a joint project supported by the EU CF network and the US CDC. Laboratory performance to correctly genotype improved over time. Reports are reviewed for content and accuracy of their interpretation. The schemes demonstrated that reporting of laboratory results varied considerably. However, over the years, the scheme revealed improvements in certain aspects of reporting including how patient and technical information is presented. Unfortunately, more than 30% of the laboratories still make at least one mistake in a submitted report .
Taking into account the different reporting policies (country specific issues), this evaluation study also provided information on the large variation between laboratories in reporting genetic testing results for carrier testing of individuals with a positive family history of CF, or for genetic confirmation testing of the clinical diagnosis of CF.
Overall, there is a high variation in the format, the content, and the quality of the written reports for molecular genetic tests. This can be improved by preparing and using consensus guidelines for genetic test reporting.

P1186. Attitudes of Medical Personnel to Ethical Issues in Iran

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A WHO meeting of experts in genetics was convened in Geneva, Switzerland, in december 1997, to review proposed international guidelines on ethical issues in medical genetics and genetic services. The medical application of genetic knowledge must be carried out with due regard to the general principles of medical ethics; ***Autonomy***, ***Beneficience***, ***Non-malficience***, ***Proportionality*** and ***Justice*** .
Human genetics with its advances, specially in the last two decades, has created new **ethical**, **legal** and **penal issues**.
This study was carried out with the purpose of obtaining points of view of 756 physicians, nurses, midwives and common people, with regard to ethical principles in medical genetics. The study was performed by questionary method and the descriptive and analytical assesment was accomplished on the results. The results showed that the application of these ethical principles in health care, have been observed with different views.
According to these views, the principles were categorized based on priority of acceptance:
1- Proportionality; 2- Beneficience; 3- Justice; 4- Autonomy and 5- Non-maleficience.
Analytical assesments suggest that a number of personal, cultural and social variables were taken in to account and the relationship between negative views and the variables were assessed.
Autonomy, with age and marriage statues, have shown statistical significance. More adults than middle aged, and more singles than married individuals, responded negatively. For beneficience, the variable of profession has shown statistical significance.
Non-malficience and proportionality showed to be not statistically significant by the variables.

P1187. A Report Prepared for Those Referred to Medical-Genetic Counseling Center of the Social Welfare Organization of Isfahan

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The medical genetic -councelling center of Isfahan welfare organization, Isfahan, Iran (Islamic Republic of).

This report contain a review and statistics for the individuals who referred to Medical-Genetic counseling center in the province of Isfahan, taking into consideration the different kinds of disease recorded in their files. This review is accomplished in a retrograde form and using the medical files of those persons referred to the above center.
All the disease recorded in their files whether related to those referred to the center or their family has been taken into consideration.
The review of the total numbers of disease out of 4400 files has come up with 24546 cases as folows.
1) The most common disease found in their files is MR. whether has been mild, moderate, severe or profound. The number is 3575 which means 14.6% of total.
2) Movement disorders: number is 2331 cases (9.5%)
3) Eye problems: number is 537 (6.3%)
4) Hearing problems: 1322 case (5.3%)
5) Speech disorders 1115 case (4.5%)
6) Sexual disorders in men and women (3.8%)
7) Physical problems: 889 case (3.6%)
8) Multifactorial disorders

P1188. A Report Prepared for Those Referred to Medical-Genetic Counseling Center of the Social Welfare Organization of BandarAbbas

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This abstract includes a review of referrals during a two years period to BandarAbbas Medical-Genetic counseling center in the south of Iran.
This review is accomplished in a retrograde form and using the medical files of those persons referred to the above center.
There is a total of 563 files which have been categorized as follows:
1) Pre-pregnancy counseling: 242 cases (43%)
2) Hemoglobinopathies: 156 cases (27.7% includes minor thalassemia, sickle cell anemia and other Hemoglobinopathies).
3) Mental and physical disabilities: 133 cases (23.6%)
4) Recurrent Abortion: 11 cases (2%)

5) Neural tube defects: 5 cases (0.9%)
6) Congenital heart diseases: 2 cases (0.3%)
7) Others: 14 cases (2.5% includes infertility, hypothyroidism, ∑)

P1189. The Dilemma of Genetic Councelling in Arabic culture: example Kuwait

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Counseling families in Kuwait which is part of the wider Arabic culture, with its rich historical, traditional and religious background still a difficult task. Our culture has its own specifity in the marriage system which is mostly consanguous and polygamy. (Consanguous marriage is about 56%) The family size is large with average 5 to 6 children which give the opportunity for genetic disorder to float on the surface. All these make the people in this region of the world unique from the genetic analysis point of view. In this paper I will discuss some family pedigrees with complicated inter-marriage system that will lead to difficulty in genetic counseling and risk calculations especially with the shortage of some facilities needed to perform proper risk calculation which increases counseling difficulties.

P1190. Four metabolic disorders in a genetic isolate: population screening and disease prevention

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The Druze in Israel is a unique ethnic community that lives in cultural and geographical isolation in northern Israel and presents high level of consanguineous marriages. We have identified four rare Inborn Errors of Metabolism (IEM) in a Druze village including Cerebrotendinous xanthomatosis (CTX), Prolidase deficiency (PD), Argininosuccinic aciduria (ASA) and Carbamyl Phosphate Synthetase I Deficiency (CPSD). The causative mutation of each disease was identified. Affected individuals were found homozygous for these mutations. These diseases represent a major cause of mortality and morbidity in the village. The aim of this study is to develop an effective carrier screening strategy to reduce the prevalence of genetic disorders in this population. We have screened 430 random healthy individuals for 4 causative mutations and found 51 carriers of CTX (1/8), 18 carriers of PD (1/24), 12 carriers of ASA (1/36) and 8 carriers of CPSD (1/54). Nine individuals were carriers for both CTX and PD (1:48). Thus, the 80 identified carriers represent a combined carrier frequency of 1/5 for these 4 rare metabolic disorders. Our results indicate that carrier screening for IEM is warranted and medically indicated in this village.

P1191. Genetic Counseling in Repeated Pregnancy Loss

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Background: Repeated pregnancy loss can be defined as the unexpected and unplanned spontaneous loss of a pregnancy before the fetus is capable of extra uterine survival for three or more clinically recognized pregnancy. A proportion of cases with RPL are caused by chromosomal, single gene, and multifactorial defects.
Material and methods: A genetic counseling program was performed for 154 couples who were referred for RPL at research and clinical center for infertility and mother hospital of Yazd, Iran. familial pedigree analysis and clinical, paraclinical and cytogenetic studies were done.
Results: We studied 154 couples with RPL. There was positive familial pedigree of pregnancy loss in 134 couples. We divided them in 4 groups based on pedigree pattern:
1- RPL in 2-3 generations (12.3%)
2- At least 2 or more familial marriage with RPL (16.2%)
3- 1 or 2 other pregnancy loss in pedigree (58.4%)
4- Negative pedigree for pregnancy loss(13%)
Our data showed that RPL was more in female relatives (51.3%) compare to male relatives (27.9%). among 82 couples chromosomal abnormality was found in 15.1% of them.
Conclusion: This study can help physicians and genetic counselors to realize the contribution of positive familial pedigree, inheritance pattern (probability of single gene disorder) and chromosomal abnormalities to cases with RPL. Familial marriage was effective in expression of RPL. (P=0.00; odds ratio=2.58).

P1192. A study of the level of young Iranian couples' knowledge about genetic counseling and genetic counseling indications, in Tehran in 2004

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The objective of this study was to know the level of young Iranian couples' knowledge about genetic counseling and genetic counseling indications in Tehran. Because of many factors for example consanguinity marriages & genetic counseling prior marriage is important in Iran.
In this study 275 couples (550 persons) have chosen and many questions have asked from them. The method of this study is descriptive-analytic cross sectional type.
The results:
1) The mean age of women was 14 and the mean age of men was 18.
2) The level of education in these cases were:
14.4% low educated
40.7% guidance & diploma
44.9% university level
3) The level of education in these cases were:
14.4% low educated
40.7% guidance & diploma
44.9% university level
4) Educational subject in these cases were:
-4.9% Medical and Health
-95.1% Non-Medical & Health
5) Hereditary disease history: 5.1% of cases had positive history.
6) 66% of cases had heard about genetic counseling.
7) 25.5% of cases had heard about genetic counseling urgency in consanguinity marriages.
8) 17.8% of cases had heard about genetic counseling urgency in positive history of hereditary disease.
9) 15.6% of cases had heard about genetic counseling urgency in positive history of hereditary disease in their families.
10) Totally, the level of knowledge in females was higher than males.
The main conclusion is the importance of increasing the knowledge of people about genetic counseling in Iran.

P1193. PUBLIC AWARENESS TOWARD GENETIC COUNSELING, DNA TESTING AND PRENATAL DIAGNOSIS. A survey from Italy.

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Genetic and congenital disorders have become the first cause of infant mortality and morbidity in the industrialised countries.
In the last decade the advances in understanding the molecular basis of many genetic conditions has created an increased possibility of early prenatal diagnosis and pre symptomatic tests. Preconceptional genetic counselling and prenatal diagnosis has increasingly been requested by both practitioners and the public at large and, consequently, important ethical and psychological problems have been raised. To better understand these complex fields it is important therefore to know the level of public awareness towards genetics.
In our study, we have chosen to administer a questionnaire to 300 Italian women to investigate the public awareness toward genetic counselling, DNA testing and prenatal diagnosis and to explore the psychological aspects influencing reproductive decisions.
Topics covered by the questionnaire were: knowledge about genetics in general, existence of genetic services, source of information, personal perception of the severity of some genetic disease, type of decision on prenatal diagnosis or pregnancy termination in an

hypothetical situation.
The results of the present study (168 questionnaires) pointed out several specific aspects, but in general indicate two main points: 1) womens'attitude toward the ethical aspects of prenatal diagnosis and pregnancy termination is quite heterogeneous; 2) more accurate and diffuse information of the public and an adequate education of professionals, including general practitioners, nurses and specialists, is absolutely required to help couples in their reproductive choice.

P1194. Development of an education programme in a thematic research network for genetic rare diseases (INERGEN)

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INERGEN is a network for co-operative research funded by FIS (Fondo de Investigación Sanitaria . Instituto de Salud Carlos III. Ministerio de Sanidad. Spain) connecting 8 research centres, with a total of 29 research groups and 204 researchers.See the presentation of INERGEN in a specific communication.
Genetic rare diseases is a complex and continuously evolving field, We have therefore considered it was very important to promote a permanent flux of knowledge, not only intranet, but also spreading it extranet in order to reach a wider social projection in benefit of the patients and the affected families, In this sense we have designed a fluid structure comprising:
a) A 3erd level of excellence, devoted to improve education and training of researchers.
b) A 2ond level for continuous education of professionals, not only in the Hospitals but also at primary care level, together with the promotion of training for genetic nurses.
c) The offer a working platform to the national patient's associations in order to know better its needs .
Planning: 1erst year. To establish the inventory of the network's resources as well as the platforms with primary care physicians and patient's associations; approval of the thematic seminaries for researchers and the offers for short stages.
2on year. Two seminaries on Bioinformatics and Genetic Epidemiology; a Cours on GRD at the "Escuela Nacional de Sanidad"; definition of the primary care and genetic nurse programmes and beginning of this activities. 3rth year, continuity of activities and organisation of a master in Clinical Genetics.

P1195. How to improve genetic counselling by improving for-client letters

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Genetic counselling should help clients to arrive at valid decisions concerning health-related problems with a genetic component. However, by the increasing cognitive complexity of the counselling process and its fragmentation over time the concept of informed consent comes under pressure. Is support of the dialogical phase by means of communication media helpful to provide enough integration of the entire counselling process? We have studied effectiveness, scope, and ethical significance of enhancing the flow of communication by for-client letters. An established format for such letters ("standard letter") was enriched systematically ("enriched letter"), based on a tape-recording of the counselling session. Standard and enriched letters were randomly and double-blindedly given to clients and compared with respect to their demonstrated comprehensibility and use value for clients, both by quantitative methods (interview and questionnaire) and qualitative methods (hermeneutic interpretation of structured interviews). Our counselling cases come from three diagnostic conditions: Suspected hereditary cancers, anomalous findings in prenatal diagnosis and infertility disorders. Altogether we counselled 142 families/couples. Analysis revealed that clients who receive enriched letters have a significantly better knowledge and comprehension of relevant facts. Qualitative analysis of 30 clients reveals the following trends: (1) Clients use their for-client letter as a private repository of information that they use for rehearsing their own case. (2) Clients find the enriched letter better accessible and more informative than the standard letter. (3) Both letter formats are subject to the following information-gap tendency: "Medically curious" clients profit from for-client letters more than "medically uninterested" clients. Supported by BMBFFKZ01KU9904

P1196. INERGEN: A Network of Research Centres for Genetic Rare Diseases

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INERGEN is a thematic network of co-operative research funded by FIS(Fondo de Investigación Sanitaria.Instituto de Salud Carlos III. Ministerio de Sanidad).
Objectives:To establish and develop a sustainable model of virtual institute for the comprehensive management of genetic rare diseases, by using different strategic programmes interconnecting traslational research to clinical applications, focused in the patients and their needs and having the analysis of the current situation of Spain as the start point.
Current resources:
8 Research Centres, with a total of 29 research groups and 204 scientists.
8 Bio-banks, containing 61,350 DNA samples;2,600 cell lines;655,000 blood/serum samples; 3,000 other tissues .
Diagnostic facilities for Chromosomal disorders , and 200 mendelian diseases. Genetic counselling. Prenatal diagnosis. Medical consultation.
Education programmes for undergraduate and postgraduate students .6 Courses on different aspects of GRD.
Strategic plan:
It is organised in 4 programmes or Scientific Sub-projects .
Development of a scientific network of banks of DNA , cells and other biological samples.
Development and validation of diagnostic protocols, as well as the management of new technologies according to health care needs,with the aim of to improve the opportunities of diagnosis treatment and prevention for the patients and theirs families.
Traslational research. The already existing projects, will be the core of multidisciplinary activities aimed to transfer research achievements to the clinical practice.
Education and interchange of investigators. The network will promote a permanent flux of knowledge, not only intranet but also extra-net, offering continuos medical education and implementing an area of co-operation with patient's associations.

P1197. Expansion in the use and availability of FISH probes via the National Genetics Reference Laboratory (NGRL) Wessex

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The aim of the Key Locus service within NGRL (Wessex) is to identify, obtain, bank and validate FISH probes from a variety of sources. In addition we are also able to provide a diagnostic service through the Wessex Regional Genetics Laboratory utilising these FISH probes (Further details and an updated list of probes are available at www.ngrl.org.uk/Wessex/probe.htlm). We are targeting a variety of rare micro deletion syndromes with probes that are not available commercially. To date, FISH probes have been tested and validated for 22 different genes/syndromes including, Mowat-Wilson, Grieg cephalopolysyndactyly, *FOXL2* (BPES), *SHOX* (Leri-Weill, short stature), Pelizaeus-Merzbacher, Soto, *TRPS1* and *EXT1*

(Langer Giedeon), a contig of PACs covering rare 22q11.2 deletion syndrome, *JAG1* (Alagille), *PAX9* (Oligodontia, Hypodontia), *EXT2* (Multiple exostoses). In addition, using a series of BACs derived from the 1Mb clone set (www.ensembl.org/homo_sapiens/cytoview), we have characterised 6 different chromosome rearrangements. These included, two rare Xq chromosome rearrangements, which were shown to be tandem duplications rather than insertions; a simple translocation between chromosomes 13 and 19 which was shown to be balanced rather than unbalanced; a query euchromatic variant 16 that had a duplication of 16p12.1 and an inverted, duplicated, and deleted 8p rather than a straightforward duplication.. In total 197 probes have been produced and validated in the past 15 months. Examples of results on a number of cases so far examined will be presented.

P1198. Education Genetics - 3 year's experience in a Genetic Service

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The Service of Genetics of the Centro Hospitalar Vila Real - Peso da Régua, S.A. is characterized for being an open service to the community.
The Service receives study visits of Secondary School and University, making possible the frequency of a concentrated theoretical and practical training programme. The purpose of these training programme is to provide to students information and some experience on the diferents aspects of clinical genetics and cytogenetics. In the clinical area, the students have the chance to attend the preconception, dysmorphology and prenatal diagnosis consultations that include ultrassonography and amniocentese. At the laboratory, they can perform cell culture techniques, chromosome preparation and staining to evaluation and image analysis.
The training programme are inserted in Science in the Holidays for Young People of the National Agency for Scientific and Technological Culture, where each student fills an inquiry before and after the training programme.
The authors present the result of the inquiries and its conclusions.

P1199. Determinants of human geneticists' attitudes toward human embryo research compared to the general public - data from a German survey.

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In recent years numerous studies assessed (risk) perception and acceptance of new biomedical technologies. Research into factors influencing professionals' attitudes is seldom. Within the research project PICTuRE (Psychological Issues Concerning new Technologies and Research on Embryos; http://www.ivp-see.uni-freiburg.de/) we aim to explore factors influencing the attitudes toward embryo research in several samples. In this presentation we compared attitudes of German human geneticists with data of the general public.
Methods: All participants in the annual meeting of the German society of human genetics in 2003 (N=792) received a 6-page questionnaire. The items pertain to reservations and promises as well as attitudes toward human embryo research, perceptions of the status of the embryo and personal characteristics (religiousness, sociodemographic and occupational variables). 122 participants responded, yielding a response rate of 18%.
Results: Regarding the human geneticists influencing factors on a positive evaluation of embryo research are childlessness ($t=2.53$; $p<.05$), lower age ($r= -.193$), higher daily working time in research ($r= .205$), lower self evaluated religiousness ($r= -.206$) and lower daily working time in patient care ($r= -.277$). Within the general public male sex (eta= .218), higher self perceived knowledge ($r= .101$), income ($r= .102$) and lower religiousness ($r= -.325$) are linked to a positive evaluation.
Discussion: In accordance with other studies we find that the attitudes of professionals are more permissive than the public's. The results reveal religiousness to be an important determinant independent from the expertise. This underlines the importance of an interdisciplinary discourse and interchange.

P1200. Genetic testing in Italy: a survey of the year 2002

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Monitoring of genetic testing in Italy started in the eighties, on behalf of the Italian Association of Medical Cytogenetics (AICM), who reported the results of the first survey in 1987. Five additional nationwide monitorings were carried out in years 1990, 1993, 1994, 1996 and 1997, by joined efforts of AICM and the Italian Association of Medical Genetics (AIGM). The two last census, collecting data of year 2000 and 2002, were carried out on behalf of the Italian Society of Human Genetics (SIGU), founded in 1998 from fusion of AICM and AIGM. The year 2002 census put major emphasis onto the number and types of genetic tests and included the clinical genetic activity. The data were raised on-line, using the SIGU website, and the results became available by September 2003. The census collected the results from 373 genetic service centers, including 159 cytogenetic labs, 147 molecular genetic labs and 67 counselling clinics. The monitoring included 230,040 cytogenetic analyses and 164,106 molecular genetic tests. Molecular tests were found to be increased starting from 1997 by an average of 40 per cent per year. The examined genes were 290. This census has documented an impressive hypertrophy of our genetic diagnostic net with a non-homogeneous territorial distribution characterized by a gradient from North to South Italy. The results demonstrated also the need of a quality assessment network, of professional guidelines, of investing in professional training and in correct public information.

P1201. DMD/BMD carrier detection and prenatal diagnosis: reflections on sixteen years of experience.

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Mutations on the dystrophin gene cause the Duchenne and Becker muscular dystrophy (DMD and BMD) that account for 50% of all muscular dystrophies. Large exonic deletions (60%) and duplications (5%) are the disease-causative mutations in two third of affected males, whereas point mutations are responsible for the remaining cases (35%).
Over the past sixteen years, our laboratory performed the disease prevention through the carrier detection of females related to affected males and the prenatal diagnosis for women at risk. Different strategies were used according to the technological progress and the knowledge of the mechanisms involved in the disease inheritance. At present, carrier identification is carried out by semi-quantitative multiplex PCR (QMPCR) on 32 exons of the dystrophin gene for the detection of deletions and duplications; linkage analysis PCR-based is still useful for cases with supposed point mutations. Up till now, more than 1300 Italian family units were analysed and 730 prenatal diagnoses performed (387 male foetuses). Our experience in defining the carrier status by direct mutation detection in females reveals that some parameters used for the risk evaluation are under- or overestimated such as the recombination rates and the maternal and paternal germinal mosaicism recurrence. Furthermore, QMPCR allowed the identification of the mutation for many isolated carriers of DMD/BMD with high serum creatine phosphokinase levels and no affected male relative in the family. In case these women were pregnant at the time of the diagnosis, accurate genetic counselling and prenatal diagnosis were offered without performing a muscle biopsy.

P1202. Rebirthing the clinic: the interaction of clinical judgement and molecular technology in the production of genetic science.

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The paper explores the relationship between science, technology and clinical process in the context of the new genetics. The purpose of the paper is to examine the role of clinical work in the development of genetic science. In doing this we rethink the nature and location of knowledge building in medical science. Drawing on qualitative research, the paper focuses on a medical specialism, dysmorphology. Dysmorphology is the medical study of complex syndromes and involves recognition of distinctive physical features. Our findings indicate that dysmorphology represents an amalgam of traditional clinical processes and new approaches to patient categorisation using genetic profiling. But clinical work in dysmorphology is about much more than fitting patients into prefixed diagnostic categories. Rather, clinical work in dysmorphology emerges as crucial to the development of genetic classification, which is itself characterised by ambiguity, uncertainty and deferral. Specifically, we show that there are a number of clinic based technologies and techniques which are, alongside molecular technology, involved in genetic categorising and the building of classification in genetics. We examine these findings to suggest that the clinic in dysmorphology is reborn as a site involved the production as well as the reproduction of medical knowledge.

P1203. Early detection inherited high cholesterol: a campaign evaluation

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The estimated prevalence of Inherited High Cholesterol (IHC) in the Netherlands is 0.8%. Most of the carriers are unaware of this, putting them at increased risk of coronary heart disease. Availability of effective treatment makes diagnosis important. Bloodlink Foundation (Dutch patient organisation) conducted a pilot campaign using regional mass media to alert inhabitants of Nijmegen on IHC. Maastricht University evaluated the exposure to the campaign and the effects. For this purpose telephone interviews (N=3377) were carried out in Nijmegen (using a pretest-posttest design) and in two control regions. The results contributed to campaign development in a second pilot region.
Of the population of Nijmegen 34.3% reported exposure to the campaign, mostly by outdoor posters or regional papers. Respondents who participated in the pretest reported higher levels of exposure, suggesting a sensitization effect. Campaign effects on cholesterol test intention could not be demonstrated. However, there were some small intermediary effects, for instance an increased 'brand' awareness of the name Bloodlink Foundation, which was largely induced by the posters (OR=5). Additionally, perceived treatment efficacy of IHC was enhanced and perceived susceptibility for IHC was normalized. Knowledge of the existence of IHC increased from 47.8% to 57.1% in Nijmegen. This increase was not attributable to the campaign; the control regions showed a similar pattern.
This study illustrates an enlarged public consciousness about inheritance, but no transfer of this knowledge into test intention or behavior. Hence, the campaign in the second pilot region has to focus on motivating risk persons to take action.

P1204. Multimedia Educational Training Program in Medical Genetics

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An e-learning infrastructure is considered essential for the delivery of educational programs. The use of a new 3D software allows the geneticians to explain the essence of the fundamental genetic phenomena. Over the past two years we have developed and updated a comprehensive web page (http://www.psisys.ro/genetica.) The Psisys Company released a free, multimedia educational kit for high school students and the interested public. The contents of the education kit are available on this website in two formats; download modules or online viewing. The students rely heavily on this page to download animations, besides using it as a source for additional reading. The animations we developed include the some processes, which we found difficult to illustrate with transparencies or in the blackboard. Followed phenomena are much better understood when shown in motion: mitosis, chromosome and chromatin structure, DNA replication and DNA repair. We developed these animations using the FLASH technology. Each stage is represented by a certain scene of the animation frame. The number of the scenes is directly proportional with the complexity of the phenomenon. Every scene can be started again until the knowledge is assimilated completely. A short explanation of the scene is inserted. The text is available newly in English, Deutsch, France and Romanian. These animations is now available on CDs for the students who have difficulty accessing them in the web.
The authors consider that the use of the multimedia programmes enhances teaching process of genetics and contributes to the computer supported training.

P1205. Iranian Genetic Counseling Network

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Missions: Iranian Genetic Counseling Network established in 1995, to recognize current status of genetic disorders, to provide official possibilities for genetic counseling all over the country, to reduce the rate of birth and costs of disabilities due to genetic diseases.
History: At the beginning counselees and clients were guided by counselors through phone. Shortly it was known that "in presence genetic counseling centers" is necessary. So, nationwide genetic counseling network designed and established thereafter. More than 450 people trained and now they are working. More than 60% of them are Physicians (general practitioner or pediatrician) and the rest of them are MSc geneticists, Nurses, and Midwives. GRC of University of welfare Science founded to support the Network in scientific, educational, diagnostic, and also research areas.
The extent of activity: Now after 7 years of activity the capitals of all provinces, and some of large or small cities have genetic counseling centers which are providing necessary information to the clients.
How do they work? They can take medical and family history, gather necessary information, construct the standardized pedigree, extract inheritance patterns from pedigree, calculate recurrence risk, request and perform necessary investigations for affected individuals and families. They can diagnose most of common and some of very rare genetic diseases. They can provide necessary information about different options regarding PND, ART, Adoption, and so on.
Priorities of the GRC and the Network, the results of more than 70000 counselng sessions will be presented.

P1206. A substitutional therapy for molybdenum cofactor deficiency

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Substitution therapies for orphan genetic diseases, including enzyme replacement methods, are frequently hampered by the limited availability of the required therapeutic substance. We describe the isolation of a pterin intermediate from bacteria, that was successfully used for the therapy of a hitherto incurable and lethal disease. Molybdenum cofactor (Moco) deficiency is a pleiotropic genetic disorder characterized by the loss of the molybdenum-dependent enzymes sulphite oxidase, xanthine oxidoreductase and aldehyde oxidase due to mutations in Moco biosynthesis genes . An intermediate of this pathway – "precursorZ" – is more stable than the

cofactor itself and has an identical structure in all phyla. Thus, it was overproduced in the bacterium *Escherichia coli,* purified and used to inject precursor Z-deficient knockout mice, that display a phenotype which resembles that of the human deficiency state. Precursor Z-substituted mice reach adulthood and fertility. Biochemical, morphological and behavioural analyses further suggest that the described treatment can lead to the alleviation of most symptoms associated with human Moco deficiency.

P1207. CETP and MDR1 SNP-haplotypes are associated with lipid lowering effect of fluvastatin in familial hypercholesterolemia

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Therapeutic response to HMG-CoA reductase inhibitors is frequently variable due to genetic differences in pharmacodynamics and pharmacokinetics. This study sought to determine whether SNP-haplotypes in the cholesteryl-ester transfer protein (CETP) and the P-glycoprotein drug transporter (MDR1) genes are associated with differences in response to fluvastatin in patients with familial hypercholesterolemia (FH).
Plasma lipids were determined following treatment with 40mg/d of fluvastatin in 77 FH patients. Four weeks of treatment resulted in mean low density lipoprotein (LDL-C) reduction of 21.48% (-22.40%-56.60%), triglycerides (TG) reduction of 8.33% (-75.58%-52.24%), and high density lipoprotein cholesterol (HDL-C) increase of 13.4% (-18.07%-46.15%). Based on 5 tagging SNPs in both genes, we reconstructed 5 and 6 common haplotypes in the CETP and MDR1 genes respectively. The association between sex- and age-adjusted standardized lipid response and haplotypes was determined by a linear regression equation. A relative increase in LDL response was associated with CETP-H13 (mean LDL-C reduction 29.38%; P=0.026) and with MDR1-h4 (26.56%; P=0.025). Similarly, TG and HDL changes were significantly associated with CETP and MDR1 haplotypes. An independent additive effect of CETP and MDR1 was demonstrated on both LDL-C and TG levels following treatment of FH patients. In conclusion, SNP-haplotypes in CETP and MDR1 have significant effects on lipid changes following fluvastatin in FH patients. The results of this study may lead to better understanding of the genetic contribution to drug response and may initiate further optimization of drug therapy. Moreover this study serves as a paradigm for analyzing pharmacogenetic effects based on pharmacokinetic and pharmacodynamic modulation.

P1208. Alendronate treatment of Brtl mouse model for osteogenesis imperfecta improves bone geometry and loading before fracture but decreases bone material quality and alters osteoblast morphology

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Bisphosphonates are widely administered to children with osteogenesis imperfecta (OI). To evaluate their effects on OI bone, we treated Brtl mice, a knock-in model for type IV OI with an α1(I)G349C substitution, and wt littermates with alendronate (Aln) (0.219 mg/kg/wk, gift of Merck) or saline placebo from 2-14 wks of age. Both mutant and wt bone had similar responses to alendronate. Total weight and femoral length were unchanged by Aln. Piximus BMD of femurs and lumbar vertebrae significantly increased. Aln improves Brtl bone geometry, with increased diaphyseal cortical thickness, a dramatic doubling of trabecular number in distal femurs, and a more rounded cross-sectional area. Mechanically, Aln significantly increased stiffness in treated wt but not Brtl femurs. The loading before fracture (yield and ultimate load) increased in Brtl, but not wt femurs. However, Aln has a negative impact on the quality of bone material. Predicted strength and elastic modulus are decreased by treatment. Femoral brittleness (post-yield displacement) was unimproved in Brtl bone and significantly increased in wt. Metaphyses of treated Brtl femurs have increased remnants of mineralized cartilage. Furthermore, osteoblast surface failed to increase in Brtl femurs, as observed in wt. Instead, fewer plump cuboidal Ob were seen in treated Brtl; many had an intermediate morphology with enlarged Golgi suggesting functionally exhausted cells. Thus, Aln treatment of Brtl bone improves bone geometry and increases loading before fracture, but decreases bone material quality and alters osteoblast morphology. These data suggest limiting duration of bisphosphonate treatment of OI bone to minimize detrimental effects.

P1209. Chaperones alleviate misfolding of cystathionine beta-synthase mutants

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Conformational diseases result from misfolding and aggregation of mutant proteins. This group of diseases may include homocystinuria due CBS deficiency. Interestingly, an incorrect folding of newly synthesized polypeptides may be prevented by various chaperones. To examine whether misfolding of mutant CBS subunits may be alleviated *in vitro,* we expressed the mutants G85R, delEx12, I278T, E176K, and A114V in *E.coli* and CHO cells in the presence of different types of chaperones, and we monitored in cellular extracts the catalytic activity and, by native western blotting, the subunit assembly.
We expressed the mutants in the presence of cellular osmolytes (chemical chaperones) sorbitol, glycerol, TMAO, and betaine. Treatment with TMAO partially restored the activity of delEx12 and A114V mutants and the use of betaine enhanced the activity and tetramerization of the A114V mutant. We examined the effect of molecular chaperones by co-expressing, in *E.coli,* the CBS mutants with GroES/GroEL. The protein chaperones partially rescued the activity and enabled a correct assembly of the delEx12, E176K and A114V mutants. We purified and characterized wild type and A114V mutant CBS polypeptides. We compared activity, UV-VIS, fluorescence and circular dichroism spectra of wild type and mutant CBS.
Our results suggest that some CBS mutants may be partially stabilized in active conformational state by an increased ligand concentration, or by chemical and molecular chaperones.
We propose that chaperones should be further explored as a potential novel treatment modality for homocystinuria- a putative conformational disease.

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P1210. Concurrent targeted exchange of three nucleotides in hprt by modified single-stranded oligonucleotides in cultured mammalian cells

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The repair of hprt point mutations by specific single stranded oligonucleotides represents a model to test targeted nucleotide exchange (TNE) in eucaryotic cells. Recently, we described the reproducible TNE of three different hprt point mutations in V79 cell lines of Chinese hamster. Here, we generated two respectively three different nucleotide exchanges in hprt in one cell by single stranded oligonucleotides. The experiments were performed in the hprt deficient V79-151 cell line showing a point mutation on cDNA position 151. The oligonucleotides resulted in mismatches to hprt on the positions 151, 144 and/or 159. In experiments using the oligonucleotides with two mismatches the mutation on position 151 was repaired as shown by genomic sequencing of clones selected by HAT. In about 11 % of these clones we detected the additional nucleotide exchange on hprt cDNA positions 159, a silent mutation. Using oligonucleotides with three mismatches in 8 % of the HAT resistant clones we generated a polymorphism on hprt position 144 in addition to the two other nucleotide exchanges. This investigation suggests that specific single stranded oligonucleotides are able to generate two or three base exchanges simultaneously in a chromosomal mammalian gene. We suggest that the mechanism

used by TNE allows the simultaneous exchange of two nucleotides in a distance of at least eight bases.

P1211. Transfection with branched polypropyleneimine (PPI) and specific 45 base modified oligonucleotides corrects a hprt point mutation in hamster cells

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Recently, we demonstrated that specific single-stranded 45 base phosphothioate modified oligonucleotides (PTO-45) are able to correct hprt mutations in hamster V79-151 cells which contain a hprt point mutation on position 151. In these experiments, the cells were transfected with linear PEI (polyethylenimine). Here, we compared the efficiency of linear PEI and branched polypropyleneimine (PPI) dendrimers of the fourth generation for the transfer of PTO-45 into the nucleus of the cultured hamster cells and human hepatoma (HuH7) cells. In these experiments, the rate of the nuclear uptake differed marginally. In V79 (or HuH7) cells it was 56% (66%) using linear PEI, and 43% (46%) using PPI 24h after transfection. Both transfection protocols showed very low toxicity. Transfection of V79-151 cells with PPI and a specific PTO-45 resulted in the correction of the hprt point mutation. The hprt conversion rates achieved did not differ substantially between PPI and PEI. This demonstrates, that PPI is an efficient carrier for transfer small single-stranded DNA molecules into the nucleus of cells in vitro and may represent an alternative agent for transfecting cells with small oligonucleotides. In fluorescence microscopy we detected a different intracellular distribution pattern of PTO-45 enhanced by PPI compared to PEI. To get further information of the uptake mechanism we performed electron microscopy studies additionally. Staining by an antibody we investigated the intracellular localization of PTO-45 molecules.

P1212. Enzyme replacement therapy for MPS I - follow up of two patients

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ENZYME REPLACEMENT THERAPY FOR MPS I - FOLLOW UP OF TWO PATIENTS

We present the one-year follow up results of two MPS I patients on enzyme replacement therapy with rh α L-iduronidase (Aldurazyme). In the first patient, a 9 year old boy, we observed significant progress in mobility and general physical condition. We also noticed improvement of hearing and visual acuity, and reduction in spleen and liver size. The symptoms and signs of sleep-apnea syndrome disappeared. Cardiac function (NYHA II) remained stable. The leukocyte α L-iduronidase activity reached therapeutic values. The second patient, an 8 year old girl, demonstrated similar improvements in the first six months of therapy: the amelioration of the respiratory function, hearing, and visual acuity, and a reduction in spleen and liver size. The sleep-apnea episodes disappeared. However, in the last six months we have not registered further improvements, and the clinical parameters remained stationary. We conclude that long term clinical and biochemical follow up is necessary to evaluate the individual response to the enzyme replacement therapy of MPS I patients.

P1213. Disease Expression And Response To Enzyme Replacement Therapy With Agalsidase Alfa Among Females Within FOS-The Fabry Outcome Survey

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Fabry Disease (FD) is an X-linked lysosomal storage disorder with severe manifestations in hemizygotes, but with variable expression in heterozygotes. FOS-the Fabry Outcome Survey, is a European database, gathering data on the natural history of the disease and outcomes on Enzyme Replacement Therapy (ERT) with agalsidase alfa. Of the 504 patients registered in November 2003, 246 (49%) were female. 43% of female patients receive ERT. The most frequent mode of presentation in females was through an affected family member. The database recorded involvement in a total of 15 organ systems. A modification of the Mainz Severity Score Index (MSSI) was calculated. In females this correlated with number of affected organs and inversely with Health-Related Quality of Life (HRQoL). The prevalence and age of onset of many features was similar in males and females, including fatigue, neuropathic, auditory and abdominal symptoms. In other respects the pattern of disease differed: progression to end-stage renal disease in females was rare (3%), yet mild renal impairment and proteinuria were common (41% and 27% respectively). Prevalence of left-ventricular hypertrophy and cerebrovascular disease in females was similar to males, but the age of onset was later. MSSI and left ventricular mass increased with age, while HRQoL and renal function declined with age. Kaplan-Meier analysis of survival in affected female relatives of patients in FOS demonstrated median age of death of 70yrs (Males 52 yrs). Female patients on ERT demonstrated significant improvements in renal function, left ventricular mass and pain-related aspects of quality of life.

P1214. Transfection capacities of some novel peptide - based gene delivery systems

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The main obstacle to achieve therapeutic effect in gene therapy trails is the problem of functional gene deliver into certain amount of target cells. Non-viral synthetic peptides are considered as perspective tools in gene delivery systems. The report deals with original studies devoted to transfection capacities of a number of peptide-based DNA- vehicles: dendritic polylysines modified with palmitoic acid residues; linear lysine-rich peptides modified with capric acid residues, and linear peptides containing HIV TAT protein motifs, enriched with RGD sequence. All DNA- vehicles were tested for their ability to bind DNA, to protect it from enzymatic degradation, and their transfection capacities in vitro. It was shown, that modification of dendritic polylysines and linear lysine-rich peptides with fatty acids residues increases their transfection and endosomolytic capacities. Maximal level of marker gene expression in vitro exceeded 25%. Transfection efficacy of TAT-peptide based DNA-carriers is at direct relation with amount of TAT motifs in it's structure. Moreover, TAT-peptide vehicles modified with RGD motifs were shown to provide the highest transfection efficiency. Thus modification of structure and composition of peptide DNA-vehicles results in considerable increase of transfection efficiency and may become a perspective tools in development of effective modular DNA vehicles.

P1215. Update on clinical trial results in MPS VI

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Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disease caused by deficiency of N-acetylgalactosamine-4-sulfatase (ASB), leading to multiple organ and tissue disease. Weekly treatment with recombinant human ASB (rhASB) has been studied in two clinical trials: Seven patients participated in a randomized, double-blind,

two-dose Phase I/II study and 10 patients participated in a Phase II, open-label, single-dose study. Week 96 data for the Phase I/II study and week 48 data for the Phase II study are presented here. All subjects tolerated infusions well and 15 continue on treatment. There were 17 SAEs, 1 drug-related, and 1 possibly drug related. Although some rhASB antibody production has been noted, it has not significantly interfered with reduction in urinary glycosaminoglycan (GAG) excretion. Biochemical response was documented by sustained reduction in urinary excretion of glycosaminoglycans (GAGs) by more than 70%. Improvements in endurance and mobility have been noted with mean increases of 96% in the 6-minute walk test, 14% in shoulder flexion, 139% in the 12-minute walk test and 147% in the 3-minute stair climb. In conclusion, rhASB treatment has been well tolerated with positive clinical and biochemical responses. Preliminary results for the Phase III trial will be presented at the meeting. (Sponsored by BioMarin Pharmaceutical Inc., Novato, CA).

P1216. Enhancement of nuclear export in Myotonic Dystrophy by post-transcriptional regulatory elements

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CTG trinucleotide repeat expansions in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene is responsible for myotonic dystrophy (DM). Full-length processed and polyadenylated mutant DMPK transcripts aggregate in the nucleus and are thought to trigger dominant effects by interacting with RNA binding proteins, which among other things result to the inhibition of muscle cell differentiation.
Woodchuck post-transcriptional regulatory element (WPRE) is a *cis*-acting module that can enhance transgene expression at the post-transcriptional level. It is believed that when inserted downstream of a given cDNA, WPRE may improve gene expression by increasing the RNA export and/or RNA cytoplasmic stability.
A construct was created where the WPRE sequence was inserted downstream the EGFP gene and the 3' UTR of the DMPK gene containing an expansion of 200 CTG repeats. C2C12 myoblasts were then transfected with the construct and others containing either the 200 repeats but not the WPRE sequence or only 11 CTG repeats. Preliminary results indicate that WPRE causes a shift of mutant DMPK transcripts from the nucleus to the cytoplasm and a subsequent increase in the EGFP expression. Stable transfections with the above constructs will reveal the importance of WPRE in enhancing nuclear export of mutant DMPK transcripts and its effect on muscle cell differentiation. Post-transcriptional regulatory elements can be used to develop an approach whereby mutant DMPK transcripts will be encouraged to travel out of the nucleus. Such an application will be beneficial for the study and the development of rational genetic therapies for DM.

P1217. Dynamic of Bone changes of children with Gaucher disease during 3 years treatment with Cerezyme (Genzyme)

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Bone changes were the most severe complication, which handicaped the patients with Gaucher disease. Our first experience with low doses Ceredase treatment was short and with interruption. After registration of Cerezyme/Genzyme we started ERT of 4 children with type-1 and type-3. The aim of our work was to investigate the bone changes of Gaucher patients during 3 years Cerezyme replacement therapy.
We used directly increased x-ray of different bones before and during ERT, as MRI of the femur and spine with T1 and T2 scanning. Because the severe clinical condition of the children, the treatment was with high doses about 60 U/kg/month, after the first years the doses decreased. Before the treatment 1 child with gene mutation N307S/n307S had severe and continous bone pains. 4 children with Gaucher disease type 1 were shown Erlernmeyers deformity, mielomegalia and increased intensy T1 and T2. The child type 3 with rare genetic mutation L444P/D409H had no deformity of the femur, but nonhomogen hyperintense T1 and T2. The x-rays of this boy showed generalized osteoporosis, scoliosis of the spine, deformity of the chest pectus carinatus which progress after the ERT. During the Cerezyme therapy the intensity of T1 and T2 of all children were changed against replacement to fat bone marrow without improvement in the remodeling of the femur. The longitudinal magnetic resonance imaging permitted us to better show the improvement of bone changes during ERT. 3 years are not enough to recover at all the bone changes of Gaucher patients.

P1218. Inactivation of telomerase activity by RNA interference (RNAi)

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Reactivation of telomerase activity during cancerogenesis is a common hallmark in most human cancer types. This enzyme stabilizes telomere length and contributes thereby to unlimited cell proliferation, i.e. immortality. Consequently, telomerase is an attractive molecular target toward which to direct cancer therapeutic agents. RNAi has recently been shown to be an effective method for inhibiting the expression of a given gene in human cells by targeting with short duplex RNA (short-interfering RNA or siRNA). Accordingly, we evaluated the ability of siRNAs to inhibit telomerase activity in several human cancer cell-lines. For our inhibition studies cells were transfected with different 21nt double-stranded RNA homologous to either the catalytic subunit of telomerase hTERT or to the functional telomerase RNA-component hTR.
At least one siRNA-molecule directed against an exon/exon junction of hTERT could be identified as most efficient in inhibiting telomerase. In comparison to control experiments this siRNA accounts for a significant reduction of telomerase activity as revealed by TRAP-assay. But, as expected, this effect was transient in duration. Subsequently, for long-term investigations human colorectal adenocarcinoma cell-line HT29 was transfected with this siRNA cloned in an eukaryotic expression vector. The resulting cells show clearly decreased mRNA for hTERT. Moreover, one subclone displays reduced proliferation rate in contrast to control cells transfected only with a nonsense siRNA-construct. Thus, the identified siRNA-molecule targeted against hTERT is qualified for in-vitro inhibition of telomerase. Comparative telomere length analysis and expression profiling could now provide new insights in the role of telomerase in the immortalisation process of cells.

EL01. Why do pregnant women decide for or against prenatal screening?

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Objectives: Prenatal screening for Down Syndrome (DS) has become a standard practice in antenatal care in many western countries. In the Netherlands, however, prenatal screening tests for congenital defects are not offered routinely. In this situation, testing is a deliberate decision because it is not part of usual prenatal care. The present study aims to assess test uptake in a large, unselected population, and to give more insight in the decision for or against prenatal screening.
Methods: The study is part of a randomized controlled trial with two different prenatal screening tests being offered, and one control group. Pregnant women receive a postal questionnaire after the test offer, with open-ended questions. 2203 women filled in the questionnaire.
Results: Uptake: 55% of women being offered the nuchal translucency measurement, and 38% of women being offered the triple test. This is considerably lower than other studies report. Main reasons for declining a screening test were 'problems with the test characteristics' (43%), 'there is no indication' (39%), 'testing will cause uncertainty/anxiety/unrest' (38%), 'not wanting an invasive test' (34%), and 'being against abortion' (16%). The reasons for accepting a screening offer were 'test characteristics' (17%), 'increased risk of

having a child with DS' (15%), 'knowledge about the health of the fetus/curiosity' (51%).
Conclusions: Our results show reasons for or against prenatal screening in strikingly different distributions than reported elsewhere. More women declined a screening offer for other than religious or moral reasons. Motivations for accepting prenatal screening were more ambiguous.

EL02. Antenatal haemoglobinopathy screening: The role of faith and religion

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The Department of Health in England and Wales is committed to implementing an effective and appropriate antenatal and neonatal screening programme for Sickle Cell and Thalassaemia Disorders. At risk couples who are offered prenatal diagnosis and/or the option of whether to continue with the pregnancy, are faced with decisions involving difficult and complex choices, where they draw on a variety of different frames of reference. These include their understanding of haemoglobinopathies but also broader aspects of their identity such as cultural values, beliefs, and family attitudes. As part of this, faith and religion are important in making sense of a couple's decisions about screening, pre-natal diagnosis and termination of pregnancy. This study explores the influence of religion and faith on understandings of screening and decisions about pre-natal diagnosis and termination of pregnancy in 12 focus groups. These include 4 male and 4 female community groups of (1) Pakistani Muslims, (2) Indian Hindus, (3) Indian Sikhs, and (4) African-Caribbean Christians, plus 2 male and 2 female groups of parents of children with (1) sickle cell disorder and (2) thalassaemia major.
This paper will present findings showing how religion and faith are drawn on in people's decisions about screening, testing and termination, and how these are inter-related with other influences, including the opinions of family members, perceived social attitudes, experiences of the conditions, and perceptions of severity of the condition. The extent to which the various influences on the decisions described are similar between the different faith communities will also be presented.

EL03. A fourteen-year social, ethical and technical struggle toward creating a model to prevent β-Thalassemia in Iran

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For fourteen years Iranian scientists have endeavored to develop a national thalassemia prevention program, aimed at controlling ß-thalassemia in Iran. According to Islamic regulations, abortion was unethical; however, great efforts led to the clerical approval of therapeutic abortion of cases with major type of β-thalassemia in 1997. An efficient nation wide prevention program with screening, counseling and prenatal diagnosis networks then developed. Full insurance coverage and nationwide educational programs have been instrumental in the efficiency of system.
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 it was possible to define only one allele: in 30 of these the diagnosis was trait or normal, while in 10 it was trait or major. We were unable to provide 8 cases with any diagnosis, corresponding to 0.88%. Our data supports the functionality of Iranian ß-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.

EL04. Multi-Center Study Shows Utility of the Beck Depression Inventory II (BDI-II) in a Population of Advanced Maternal Age Patients (AMA) Patients

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Depression is a serious psychiatric illness that alters mood, cognitive and physical functioning, and impairs day-to-day functioning. Depression affects individuals of all social strata and ages. Most healthcare providers are aware of post partum depression, yet depression during pregnancy appears equally prevalent. Depression in pregnant women can affect understanding, perception, and decision-making ability. It is also associated with pregnancy complications such as low birth weight, prematurity, and preeclampsia. With experience in medical genetics and counseling, genetic counselors are health professionals in a unique position to identify prenatal patients with depressive symptoms. Genetic counselors routinely perform psychosocial assessment. Since depression may adversely affect attention to and comprehension of critical medical information by pregnant women during genetic counseling, it is imperative that genetic counselors recognize depression. To determine whether current genetic counseling practices adequately detect clinically significant symptoms of depression in prenatal patients with AMA, a multi-center study was conducted. Sixty-eight AMA patients completed the BDI-II and were also assessed for symptoms of depression by prenatal genetic counselors. The BDI-II detected depression in 15 of these patients. Genetic counselors identified depression in 2 of these 15 patients during their traditional assessment. The sensitivity of the genetic counseling assessment was 0.13 percent, with a specificity of 0.95 percent. Traditional prenatal AMA genetic counseling may not be sufficient to detect subtle signs of depression. Incorporation of a depression inventory may be useful in identifying those in need of further evaluation and treatment, and ultimately enhance prenatal and postnatal outcomes.

EL05. Generating narratives after an adverse result from prenatal diagnosis: program evaluation

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We have defined an intervention program, based on cognitive-narrative therapy and the Ottawa decision framework, to prevent problems in decision-making and adjustment when terminating a pregnancy (TOP), after an adverse result from prenatal diagnosis. The program was implemented with 24 women and the results compared with a control group of 67 women, who also terminated pregnancy but without intervention. Among all, 64.4% had DNA or karyotype analysis, and 35.6% an ultrasound; mean age was 33.0 years, and mean gestational age was 19 weeks.
The intervention had 4 sessions: (a) decision; (b) subjectifying; (c) metaphor, and (d) projection session. Its aims were to prevent depression and anxiety after TOP, achieving episode narratives with multiple meaning and coherences. There were also two evaluations, at the 15th day and the 6th month after TOP (using Beck Depression Inventory, Zung Anxiety Scale, Perinatal Grief Scale and a specific instrument to assess the homogeneity of the intervention program). The results showed that subjects who went through this intervention program had significantly less anxiety and depression at 6 months ($p<0,05$), despite of no differences at the first evaluation. We also reviewed cases for a negative evolution: 22.9% and 19.6% of the control group had an increase of anxiety and depression levels, while in the program group these values were only of 9.1% and 0%, respectively.
We conclude for a positive impact of applying this intervention program and the importance of the meaning-making process in a paradoxical experience as is the termination of a desired pregnancy.

EL06. Does offering prenatal screening influence the psychological well-being of pregnant women?

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In the Netherlands opponents to offering prenatal screening to all pregnant women argue that screening leads to anxiety and influences the attachment of the mother to her unborn child. This study aims to give insight into the psychological effects of testing.
The study was a randomized controlled trial. Women were offered the nuchal translucency measurement or triple test, or were randomized in the control group. They filled in 4 questionnaires during pregnancy and 1 after delivery. Data of 1400 women were used.
Anxiety was measured by the Dutch version of the Spielberger State-Trait Anxiety Inventory and the Pregnancy Anxiety Questionnaire. Attachment was measured by a self-developed questionnaire (10-point scale).
Offering prenatal screening did not seem to influence the level of general anxiety in women (no significant differences on STAI-state scores), but women who chose to have prenatal screening done, were already more anxious about the health of their child before information about screening was given compared to women who chose against screening ($t = 5.1$ $p < 0.05$). After a negative test result these anxiety levels decreased to the level of the control group. Positively screened women ($n = 29$) were more anxious than negatively screened women after they got the test result ($t = 4.0$, $p < 0.05$). This difference disappeared in the 3rd trimester of pregnancy. Attachment to the unborn child increased with pregnancy duration, but offering screening did not influence this development.
In conclusion, offering prenatal screening doesn't seriously affect the psychological well-being of pregnant women.

EL07. Stigma in ectodermal dysplasia narratives

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X-linked hypohidrotic ectodermal dysplasia (XHED) manifests with sparse hair, absent or malformed teeth and reduced sweating that causes heat intolerance. It can be a life-threatening disorder, especially in early childhood, while it may later be regarded as primarily cosmetic. The experience of living with XHED has been discussed in more than 20 interviews with affected males and/or their mothers. We report the content of these interviews in terms of the identity work performed by affected individuals to cope with the stigmatisation they often experience. We also examine the use of reported speech, thought and feelings in these interview narrative accounts. Reported speech often serves to emphasise both the predicament and the coping skills and strong character of the affected individual. It can also be used when reporting moments of strategic decision and when introducing especially sensitive topics.
One context in which the strength (or degree) of stigmatisation can be made manifest is in discussions about reproduction and the 'risk' of transmitting the condition to the next generation or the 'guilt' at having done so. The relative weight given to social factors as opposed to physical difficulties can give insight into the perceived burden of stigmatisation in this condition. In addition, the reporting of information or advice as coming from health professionals conveys something of the impact of the professionals' opinions and judgements on affected individuals.

EL08. Turner Syndrome: Four Challenges Across the Lifespan

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Turner Syndrome (TS) is a sex chromosome anomaly that occurs in approximately 1/2500 female births. Individuals with TS have a partial or full deletion of one of the X chromosomes. In almost all cases, women with TS are infertile and short in stature. Despite the prevalence of this chromosomal condition, the challenges these women face throughout their lives are not fully understood. This study aimed to characterize the subjective experiences of individuals with TS throughout their lifespan, to investigate the concerns and obstacles faced by these individuals and their families, and to offer insight into the strengths and weaknesses of the delivery of health care as perceived by girls and women with TS. Ninety-seven affected girls and women and 16 parents participated in a qualitative interview study. Infertility, short stature, sexual development, and health were the subjects of greatest concern repeatedly cited by the majority of participants. The relative weight of these concerns tended to shift based upon the individual's age and life experiences, but all four issues remained salient throughout the lifespan. Enhanced awareness of the evolving physical and psychological challenges faced by girls and women with TS may help health care providers improve the quality of life for these individuals. Recommendations for providers are made based on suggestions from participants.

EL09. A study of the psychosocial impact that reproductive information has on young males living with Cystic fibrosis

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As more individuals with Cystic fibrosis (CF) survive into adulthood, sexual health and parenthood issues are becoming increasingly important. Most males with CF are infertile due to congenital bilateral absence of the vas deferens (CBAVD). However, assisted reproduction techniques provide hope for these men to father their own children. Few studies have focused on the emotional and psychosocial impact that reproductive information has on males with CF. This qualitative study was carried out to explore how men with CF feel about their infertility, sexuality, parenthood and assisted reproduction.
13 affected men, aged 26-47 years who attend an adult CF unit in the North-West of England consented to be interviewed. Semi-structured interviews were audiotaped, transcribed and subjected to thematic analysis.
Participants felt that males with CF should be informed about infertility issues around the age of 14, considerably younger than the average age of 19 years that these participants were informed of their potential infertility. Participants' thoughts about infertility changed over time, being most significant for men in their late 20's / early 30's. All men had heard of semen analysis and assisted reproduction but the majority did not want to know their definite fertility status or embark on an assisted reproduction programme. Fears about parenthood included the risk of having a child with CF and concern about dying and leaving a child fatherless. Men from this study did not feel that CF had a negative impact on their sex lives, although some felt their illness had directly caused relationship break-ups.

EL10. Long-term Satisfaction with Bilateral Prophylactic Mastectomy and Immediate Breast Reconstruction in Genetically Predisposed Women

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Context Prophylactic mastectomy with immediate breast reconstruction (PM/IBR) is a risk-reducing strategy for women with a BRCA1/2 mutation or a significantly increased risk of breast cancer because of family history. It remains a very radical intervention while long-term data on satisfaction are insufficiently available.
Design, Setting, and Participants Retrospective study in high-risk women participating in a longitudinal follow-up study on the medical effects of PM/IBR (n=136), performed at one institution. The questionnaire was completed by 84% (n=114) of the women.
Main Outcome Measures Satisfaction with PM/IBR and impact on the sexual relationship.
Results The median follow-up time between PM/IBR and this analysis was 3 years. Satisfaction with the outcomes of prophylactic

surgery was significantly correlated with: experienced complications, ongoing complications, not considering the breasts as ‚their own' and not choosing this type of IBR again. Forty-four percent of the women reported adverse effects on their sexual relationship. This observation was strongly correlated with perceived lack of information, discrepant expectations, ongoing complaints and limitations, negative feelings of femininity, partner's negative perception on femininity and sexuality, and not choosing this type of IBR again.
Conclusions The majority of women would opt for PM/IBR again. However, adverse effects influenced the satisfaction with the result of PM/IBR negatively. Furthermore, untoward changes in the perception of the sexual relationship as a result of PM/IBR were reported. This needs to be addressed and explored in the counseling to optimize an informed choice for the woman at increased risk, and to enable adequate adjustment after treatment.

EL11. Outcome of non-carriers after predictive testing for Huntington's disease: Short-term paradoxical reactions and long-term psychological and social adjustments

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From 1992 to 2004, 837 at-risk persons requested presymptomatic testing (PT) for Huntington's disease (HD) at the Salpêtrière Hospital (Paris). After going through pluridisciplinary counselling procedure, 393 obtained their result including 162 carriers and 231 non-carriers. To evaluate the long-term outcome we contacted 351 testees, who obtained their results between 1992 and 2001, and 118 were interviewed. The evaluation included a semi-structured interview to assess sociodemographic characteristics, coping with the results, life events and changes. This included a systematic search for current depression (self reports such as Beck Depression Inventory, Beck Hopelessness Scale, Spielberger State, Trait Anxiety Inventory and the mood section of the Mini Neuropsychiatric Interview) and its treatment. Immediately after PT, 43% of non-carriers expressed happiness, 39% had paradoxical reactions including depression and anxiety, suicidal behaviour, bad social adjustment, crisis identity, matrimonial crisis, and no defined reaction in 18%. In comparison, carriers had bad outcome (72%), well being (5%), no reaction in the remaining. After a mean delay of 3.8 years (SD 2.5), after the result, non-carriers were significantly less depressed ($p<0.01$) anxious ($p<0.01$) and hopeless ($p<0.001$) compared to carriers, but the differences were less marked than just after the result.
We illustrate our study with two cases, one man with an awakening type of reaction and a woman with suicidal behaviour after a favourable result.
Time and follow-up are necessary to recover from being at-risk, since coping with good new depends of the image built in the context of HD and the at-risk feeling.

EL12. Predictive testing for Huntington's disease: The relationship with the partner in the 5-year period after testing.

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Introduction: Predictive DNA-testing for serious, incurable late onset diseases has far-reaching implications for the tested person, but also for the partner. Psychological functioning of tested persons has been extensively described; several studies showed that, for the majority of tested persons, the personal benefits of knowing the carrier status outweigh the psychological distress after the test. The ramifications of predictive testing for the relationship with the partner received less attention.
Aims: The study focused on the partner-relationship of asymptomatic tested persons in the five-year-period after predictive testing for Huntington's disease. We describe changes in marital status, perceived quality of the relationship and perceived changes in the relationship.
Methods: Twenty-six (asymptomatic) mutation-carriers, 14 of their partners, 33 non-carriers and 17 of their partners participated in the 5-year study. Qualitative (interview-data) and quantitative methods (questionnaires) were used.
Results: For the majority of tested persons (70%), the marital status was unchanged five years after testing. Overall, carriers rated the quality of the relationship higher than their partners did and they perceived more positive changes. Qualitative data further show that some couples, carrier as well as non-carrier couples, had difficulties in finding a balance between the tested person's needs and the partner's needs.
Discussion: Hypotheses about the role of genetic risk and test result in (un)balanced relationships will be formulated in order to explain some of the findings. Implications of the findings for pre-and post-test counselling will be discussed.

EL13. Predictive genetic testing for BRCA1/2 in the UK: The long-term psychosocial impact

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Purpose: This multi-centre UK study assesses the impact of predictive genetic testing for breast/ovarian cancer gene mutations (BRCA1 and BRCA2) on mental health, perceived risk of developing cancer, and risk management following ascertainment and disclosure of gene carrier status.
Methods: 315 adults (male and female) from 9 UK genetic centres were invited to participate. Assessment was by pre-validated questionnaire at baseline (pre-test) and at 1, 4, 12 months, and 3 years following disclosure of the genetic test result. This paper focuses on the 3-year data. There were 100 gene mutation carriers and 185 non-carriers.
Results: In the 12 months following disclosure of the test result non-carriers experienced significant reductions in cancer worry. Amongst carriers, cancer worry rose in the month following disclosure of the result and returned to baseline levels at 1 year. Female gene mutation carriers engaged in significantly more risk management strategies than non-carriers in the year following testing. At 1 year 23% of female carriers reported insurance discrimination. We report levels of cancer worry, uptake of risk management options, perceived risk and insurance discrimination 3 years following the genetic test result.
Conclusions: The results clarify the issues that arise when people unaffected by cancer are offered genetic testing as part of routine clinical practice. It is important that genetic counselling and support is available in carrier clinics following disclosure of the test result aimed at improving management of medical and psychological outcomes.

EL14. Predictive genetic testing for hypertrophic cardiomyopathy (HCM) in children .

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Predictive genetic testing in children is widely accepted for conditions in which the onset of disease occurs in childhood or adolescence, and for which there is an effective treatment or preventive intervention. The request for predictive testing for HCM in children presents practical and ethical challenges to genetics professionals. Certain disease features of HCM, a common autosomal dominant condition, complicate the decision about the optimal age to offer predictive testing:
• The onset of disease can occur in childhood but frequently occurs later.
• The clinical course is usually benign, but may be complicated by cardiac symptoms and sudden death, which can be the first disease manifestation.
• The penetrance and expressivity are variable.

• Current recommendations advocate that clinical screening of at-risk children should commence at 12. In practice screening often starts before this because of parental anxiety and the small risk of sudden death in childhood.
This paper will present the clinical indications and an ethical analysis of the reasons for and against predictive genetic testing for HCM in children. These include the future autonomy of the child, parental anxiety, family well-being, and insurance implications. The potential psychological impact will also be considered, which is the topic of a pilot study we are currently conducting. It will be argued that in a climate of appropriate clinical management and counselling expertise, there are good practical and ethical justifications for undertaking predictive testing in children. A more difficult question involves whether testing is justifiable in very young children, or infants.

EL15. Genetic testing for melanoma susceptibility: Psychosocial issues for families at high-risk.

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The potential role of genetic testing in families with an inherited pattern of melanoma is a complex issue. Despite heightened public awareness, and growing interest in access to familial cancer services from those at perceived risk of familial melanoma, the psychosocial consequences of genetic testing for mutations among individuals at high-risk of melanoma remain unknown. To investigate this issue, we have commenced a multiphase, interdisciplinary study involving samples of affected and unaffected individuals at either high- or average-risk of developing melanoma due to family history. Using semi-structured individual interviews (N=40), the qualitative component of this study addressed six key issues: (1) the role of genetics in lay individuals' causal attributions for melanoma; (2) the impact of family history and disease experience on melanoma representations; (3) knowledge about, and attitudes toward, genetic testing for melanoma risk; (4) the impact of attitudes toward genetic testing on health behaviour practices; (5) the relationship between cancer-related anxiety and attitudes toward genetic testing of children; and (6) informational needs of groups of individuals at varying risk of developing melanoma. Our results reveal several important thematic differences between groups at varying risk of melanoma, particularly in terms of motivations for genetic testing and anticipated emotional responses to test results. The demand for genetic testing for melanoma risk from those at high-risk appears to be mediated primarily by the need for explanatory relief. In time, our results will provide a much-needed empirical basis for the development of guidelines for the psychosocial care of individuals at increased risk.

EL16. Hereditary melanoma and predictive genetic testing: Why not?

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Background: Since p16-Leiden pre-symptomatic testing for hereditary melanoma has become available, uptake has remained 26 %, which is low given the good surveillance and treatment options. Why are non-participants reluctant to participate in genetic testing?
Methods: 66 eligible individuals, who were knowledgeable about the test but had not participated in genetic testing by January 2003, completed a self-report questionnaire assessing causal attributions, risk awareness, family communication, motivation and anxiety.
Results: Non-participants reported anxiety levels below clinical significance. A principal components analysis of all possible reasons distinguished two underlying motives: emotional (i.e. anxious) and rational motivation. Emotional motivation was associated with unrealistic ideas of what caused melanoma, hesitation to inform the family about an unfavourable test result and with higher scores on anxiety. Rational motivation was associated with more accurate risk awareness, the inclination to have presumptions about which family members are mutation carriers and lower scores on anxiety.
Conclusion: Non-participants were not clinically anxious. Anxiety, however, was found to be associated with emotional motivation and inversely with rational motivation. Misperceptions of the causes of melanoma and family communication partly explained the reluctance to undergo testing. Therefore, more attention should be paid to information provision and to the role of family communication in the uptake of genetic testing.

EL17. How to raise the haze of Bayes - improvement of diagnostic inferences and of the understanding of risk by students and physicians is possible

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Genetic tests become increasingly accessible. Possible benefits of testing for susceptibility to chronic disease may be substantially outweighed by confusion raised by test results. The concept of sensitivity, specificity and positive predictive value of a test are all complex and therefore not necessarily understandable to an uninitiated patient. On the other Hand in order to make informed decisions on participation in genetic screening programs and in prenatal diagnosis patients should be told about these concepts. Moreover several studies have shown that even physicians have a poor understanding of probabilities and the predictive value of test results. Gigerenzer and coworkers hypothesised that due to human evolutionary development mental algorithms were not designed for probabilities and a Bayesian reasoning but for the understanding of natural frequencies.
In order to test their hypothesis for genetic counselling four problems were presented to more than 200 of our fellow students (representatives of an upper-middle class lay population) either as probabilities or as natural frequencies: (1) positive triple test and the risk of trisomy 21, (2) insulin dependent diabetes mellitus and DR3/DR4, (3) breast cancer and BRCA1/BRCA2, (4) inheritance of familial polyposis and symptom free ageing. Participants received in a randomised order all four problems, two presented as probabilities and two as natural frequencies. They generally ranked the natural frequency questions as less difficult and yielded a significant better understanding of the risk. The representation of complex concepts in natural frequencies rather than in probabilities can improve the understanding of patients and of physicians.

EL18. Impact of an information booklet on satisfaction, knowledge and decision making for BRCA genetic testing

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Objective: we investigated the impact of a standardized information booklet associated to a cancer genetic consultation on a set of outcomes including characteristics of decision-making for genetic testing.
Material : Patients included were proposed an identification of BRCA mutation for the first time in their family. They were all women cancer affected. One group was surveyed before (N1=305), and the other after the booklet was available (N2=342). A questionnaire was mailed during the month following genetic counselling. The following outcomes were measured: time allocated to genetic counselling, satisfaction (22 items), knowledge (12 items), decisional conflict (Decision Conflict Scale, O'Connor, Ottawa, 16 items), and intentions to be tested.
Results: The answer rates were the same for the 2 groups surveyed (87%). In the "booklet group", there was a significant increase ($p<0.05$) in several of the satisfaction dimensions in particular those concerning the quality of information delivered about cancer risks. There were no modifications of the perception of the doctor-patient relationship neither of the overall perceived usefulness of the consultation. Knowledge scores were significantly increased

(p<0.05). The intentions to be tested were not modified but the informative dimension of the decisional conflict scale were (p<0.05). Conclusion: A standardized written document associated to a cancer genetic consultation is an interesting complementary instrument to improve knowledge and decision making for genetic testing.

EL19. Providing psychosocial care in hereditary cardiac disorders - possible indicators of psycho-social vulnerability.

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Introduction In our department of Cardiogenetics, counselees undergoing predictive genetic testing for hereditary cardiac disorders are seen for psycho-social counselling by protocol (for families with underage children) or on referral.
Aim To describe 1) the number of psycho-social counselling sessions and 2) possible correlates, as assessed in the first session, of an increased number of sessions (≥3).
Methods From May'99 to Sept.'02, 47 counselees and/or parent couples were referred for psycho-social counselling. Sessions were recorded in an electronic data-base. Key points from the first session were: previous/childhood experiences with cardiac disorders, sudden death in nearby family, counselee's family role, and experienced burden of psychological/social impact.
Results

Counselee characteristics and number of sessions			
Counselee(s)	Age mean(sd)	Disorder (LQTS,HCM,ARVD)*	Number of sessions
Adults (single and/or no children),n=8	22.5(1.8)	2/6/0	2.1(0.93)
Parent(s) with children (1 or more under-age; all >18 years), n=30;n=4)	38.2(6.1); 57.8(10.4)	20/7/3; 2/2/0	3.4(3.1); 4.8(3.8)
Minors(<18years),n=5	14.0(2.0)	2/3/0	4.6(2.3)

(*LQTS=long QT syndrome; HCM=hypertrophic cardiomyopathy, ARVD=arrhythmogenic right ventricular dysplasia; **Parent-couples were recorded as a single case.)
Significant associations with ≥3 sessions were found with 'childhood experiences with disorder' (χ^2=10.7,p=0.001), 'sudden death in nearby family' (χ^2=4.5,p=0.03), 'having messenger-role/example role in family' (both χ^2=23.1,p<0.001), and 'experiencing psychological impact as burdensome' (χ^2=8.3,p=0.004). Compared to referrals in hereditary cancer in the department, more psycho-social sessions were provided (4.02 vs. 2.04,p=0.001).
Conclusions Important indicators for needing additional psychosocial care can be assessed early in the counselling process. Additional research will explore the content of the counselling, to identify possible helpful interventions.

EL20. A way to improve case-management in psychosocial counselling in the day-to-day practice in Clinical Genetics - a Practice-based Instrument for Predictive Testing (PIPT).

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Background Predictive genetic testing has become possible for an increasing number of disorders, each with its specific characteristics. In our work as psycho-social counsellors, we experienced a need for more standardized disease-specific guidelines in our day-to-day contacts with counsellees.
Aim Therefore we developed an instrument , aimed at registering specific psycho-social issues.
Methods We developed an MS Access database: PIPT; a Practice-based Instrument for Predictive Testing which quantifies the important psycho-social issues of counselees undergoing predictive testing. It offered us the following opportunities:

- reciprocal tuning
- standard reports
- quality testing
- data gathering

Results

- PIPT leads to mutual tuning between colleagues.
- Standard reports to referring clinical geneticist facilitates our teamwork during the process of decision making and testing.
- It shows which issues are of importance and provides a risk-assessment of the counselee's wellbeing in the near future
- Using PIPT has enabled us to improve our case-management.

Conclusions PIPT focus specifically on reporting important psycho-social information during the process of counselling and testing. This provides information on, for instance, certain vulnerable groups and duration of the counselling contacts*.
A limitation of PIPT is that our interventions are not yet recorded. Also the input of the data after each counselling takes a lot of time. We are working on an improved version, which will address the above limitations.
The opportunities and limitations of PIPT can be illustrated on the basis of a case.
*see abstract by Mollema et al, AMC, Amsterdam

EL21. Counselling Supervision for a Team of Genetic Counsellors from the UK

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'Counselling' or 'psychological supervision' focuses on the emotional and psychosocial issues raised by an interaction with a patient. These can include ethical dilemmas relating to the patient's situation, difficult emotional reactions or guidance on providing appropriate psychological support. 'Clinical supervision' is often more focused on the practical aspects of clinical management.
The Association of Genetic Nurses and Counsellors (AGNC) (professional body for genetic counsellors in the UK), suggests that genetic counsellors should participate in counselling supervision. However, there is no guidance on what form this should take.
The Medical Genetics Department, Cambridge, UK, currently employs eight genetic counsellors. Counselling supervision was set up for this team offering one hour of one-to-one supervision for each person plus two hours of group work every 6 weeks. The counselling supervisor is a qualified psychological therapist, working in a specialist palliative care setting. Within her work, she considers the unconscious processes of people and organisations/systems as documented by Bion and Foulkes. Transference and counter-transference are central to the process of analysis of material brought to all sessions, as is a holistic approach encompassing existential and philosophical ideas.
The attitudes and opinions of the team of genetic counsellors from Cambridge were documented prior to having supervision, and after it was in place. The general attitudes were very positive showing a reduction in work-related stress after the commencement of supervision. A comparison is made between the impact of one-to-one versus group supervision. Specific details will be explored and examples of case studies presented.

EL22. Pharmacogenetics: Ethical issues

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Pharmacogenetics, the study of genetic variation that affects response to medicines, has the potential to play an important role in improving the safety and efficacy of treatments. However, both the research and its application raise ethical, legal, social and regulatory issues which it is important to consider now. Issues discussed will include the application of pharmacogenetics to the research and development of medicines, including questions about the way in which clinical trials are designed and managed, and the impact on the cost of undertaking clinical trials. There are also concerns that the introduction of pharmacogenetics might lead to a further stratification of the drug market that discourages pharmaceutical companies from developing drugs that would provide a significant benefit but only

to a small number of patients. Other issues relate to the use and storage of genetic information, allocation of resources and the impact on clinical practice. One question for example is how much freedom patients should have to purchase their own pharmacogenetic tests or to receive a medicine while refusing to take the associated test.
If the potential benefits of pharmacogenetics are to be realised, consideration needs to be given to what incentives should be put in place to maximise possible benefits while also protecting the interests of patients and of society. The Nuffield Council on Bioethics published its Report on ***Pharmacogenetics: ethical issues*** **in September 2003**. The talk will draw on the conclusions made in this Report, and present recommendations for future policy and practice.

EL23. The psychological burden of diagnostic uncertainty for parents of disabled children

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Diagnostic and prognostic uncertainty is well-known to constitute a major psychological burden for parents of sick or disabled children. However, the long-term effects on the parents of having or not having a precise genetic diagnosis, in terms of emotional stress, remain unclear.
In this questionnaire study on over 700 parents, we compared mothers of healthy children with mothers of children with Down syndrome, and with mothers of children with a diagnostically unassigned mental retardation with regard to the level of anxiety (State-Trait Anxiety Inventory / STAI), feelings of guilt, and emotional burden.
While mothers of children with Down syndrome score comparably to the mothers of non-disabled children, the results show broad psycho-emotional disadvantages for mothers with a mentally retarded child of unknown diagnosis. Consequently, the value of genetic diagnosis of infantile disabilities encompasses, beyond clinical considerations like therapy planning and assignment of the recurrence risk for siblings, significant and long-lasting emotional relief for the parents. We discuss the pros and cons of presenting „working diagnoses“ instead of outspoken uncertainty to the parents of children with disabilities of unclear etiology.

EL24. Genetic professionals‘ reports of non-disclosure of genetic risk information within families

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Patients attending genetic clinics are often the main gatekeepers of information for other family members at risk. There has been much debate about the potentially conflicting responsibilities of clinical genetic professionals where patients indicate that they will not transmit important genetic information to their relatives. We report findings from the first prospective study investigating the frequency of cases of potential non-disclosure within families, which have caused concern to genetic professionals. Twelve UK and 2 Australian Regional Genetic Services reported such cases over a 12 - 18 month period, including details of the actions taken by professionals. Sixty five cases of non-disclosure were reported, which represented a very small proportion (<1%) of the total genetic clinic consultations in the collaborating Centres during the study period. These included 39 cases of parents not informing their adult offspring, 22 where siblings or other relatives were not informed of their risk, and 4 where disclosure was not made to partners. Professionals reported clients reasons for withholding information from relatives as complex, more often citing concern and the desire to protect their relatives rather than poor family relationships. In most cases geneticists reported that they, “took further steps to persuade the consultand to make a disclosure”, but in no instance did the professional decide to disclose information without the consultand’s permission.
This was a multi-centred collaborative un-funded study carried out by clinicians and academic colleagues, and the benefits and drawbacks of this approach to research into clinical practice will be highlighted.

EL25. Experience of Growing up with a Sibling affected by Down syndrome and Reproductive Choice

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Growing up with a sibling affected by Down syndrome is reported to have varied effects on an unaffected sibling. This study aimed to enable adults to express their views on their experience of growing up with a sibling affected by Down syndrome. Although these individuals are not generally at increased risk of having a child with Down syndrome, their perception of this risk and their views on having children and on having screening for Down syndrome in a pregnancy were explored.
Semi-structured interviews were undertaken with seven individuals who have / had a sibling with Down syndrome. Transcripts of these interviews were analysed to identify, compare and explore themes that emerged.
The impact of having a sibling with Down syndrome varied from positive life-enhancing experiences to psychologically challenging experiences. The differences are discussed and suggestions made to enable parents and professionals recognise and reduce potential problems.
Two participants thought that their risk of having a child with Down syndrome was increased due to their sibling having Down syndrome. Factors in the decision to opt for screening for Down syndrome in a pregnancy included the perceived impact of having a child with Down syndrome, the perceived benefits of screening, and the perceived risk of having an affected child. Participants own experiences influenced the perceived impact. The main factors influencing the hypothetical decision to keep or terminate an affected fetus, were the perceived impact and the opinions of significant others.

EL26. Psychosocial counselling of healthy siblings in a genetic clinic.

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We have been concerned how the healthy siblings cope in families with rare disorders. There are very few studies on this subject. In the following we wish to bring into focus various experiences and problems related to the counselling of children and young people with a rare disorder in the family. We carried out a preliminary study offering counselling to siblings in 14 families, 7 of which accepted to take part in the study. From these families 8 healthy siblings had one counselling session and one was counselled twice.
The diagnoses in these seven families were: mild mental retardation of unknown reason (3), rare chromosomal disorder (2), neurofibromatosis type 1 (1), and aspartylglucosaminuria (1). The ages of the healthy siblings ranged from 7 to 16 years. The counselling session with a genetic nurse lasted for about an hour. Some of the siblings also met a medical geneticist together with the rest of their family. It is our intention to evaluate the content and methods of counselling as well as to consider its benefits and problematic areas.
Our preliminary study indicates that great emphasis should be laid on the choice of counselling method to comply with the child’s age. We noticed that if the children had been prepared already at home for the counselling, they were far better able to talk openly and thoroughly. It seemed that it was very important for the healthy siblings to have a counselling appointment of their own with the undivided attention of the genetic nurse.

EL27. Patients’ understanding of their family history of common chronic diseases

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Background The literature suggests that there is a divergence between the ways in which individuals with a family history (FH) perceive their risk and the risk estimates provided by health professionals. However previous research, particularly of people with a FH of cancer, may have been influenced by the genetics clinic setting. This study aimed to explore the meaning of their FH to a primary care population.
Method Thirty semi-structured interviews were conducted with adult primary care patients from Cambridgeshire, who had a first degree relative with at least one of the following illnesses: cancer, heart disease or diabetes. A constant comparative approach was adopted to analyse the interview transcripts.
Results The findings indicated that the development of perceived personal vulnerability to the illness in the family depended not only on the biomedical model of counting affected relatives, age and cause of death, but also on a sophisticated interplay of other factors. The emotional impact of witnessing illness in the family, details of the trajectory of the relative's illness such as sudden, premature, prolonged or fatal illness, and concepts of emotional closeness and likeness of personality or mannerisms with the affected relative, all contributed to perceiving the illness in the family as serious and salient.
Conclusions This study highlights the variations between lay and professional understanding, and demonstrates the importance of eliciting the patient's perspective. The elucidation of patients' understanding of their FH will inform the development of patient-centred genetic risk assessment, enable better risk communication, and support informed decision making.

EL28. Implications of genetic risk information in families with a high density of bipolar disorder: An exploratory study

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While major susceptibility genes for bipolar disorder are yet to be identified, the opportunity exists to ascertain systematically the important issues and societal implications of genetic risk determination for bipolar disorder prior to these technological advances becoming widely available. This study explores, in a sample of families with a high density of bipolar disorder: (i) attitudes to predictive genetic and prenatal testing, using different risk frames; (ii) attributions for bipolar disorder, in particular the degree to which a genetic model is endorsed; and (iii) the impact of these attributions on the perceived stigma of bipolar disorder. A qualitative methodology was selected as most appropriate as no previous research has examined this issue. Participants were ascertained through a molecular genetics study of bipolar disorder. In-depth interviews were conducted with 21 members of families with a high density of bipolar disorder. Most participants reported being interested in genetic testing if it gave a definitive answer, while expressed interest in testing was lower if it gave a probable answer only. Almost all stressed that a genetic susceptibility and environmental factors interacted. Most participants felt that a genetic explanation was likely to decrease the stigma associated with bipolar disorder as it shifted the locus of control and responsibility away from the individual towards the role of heredity. Findings indicate that expressed interest in genetic testing depends on the certainty imparted by the test. Results suggest that families with bipolar disorder are likely to benefit psychologically from information about the genetic basis of bipolar disorder.

EWS01. Multidisciplinary genetic clinics for individuals with genetic syndromes and developmental delay.

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Many individuals with developmental delay have special medical needs related to for instance congenital malformation(s), associated neurological disease such as epilepsy or sometimes an increased risk of cancer as seen in phacomatoses. A close interaction between the different medical specialties clearly improves the medical care of the individual, and for this reason, many centers have created multidisciplinary clinics for specific syndromes such as Neurofibromatosis, Down syndrome, Velocardiofacial syndrome and Williams syndrome. At the same time, many genetic syndromes are associated with a recognisable pattern of development and behaviour. Besides its importance to clinicians as an aid to syndrome diagnosis, knowledge of this behavioural phenotype and its remediation
offers immense direct practical value to the management of the child. Therefore, professionals such as educational psychologists are essential in these multidisciplinary teams.
Likewise, genetic and hereditary disorders have major psychosocial and familial implications, which can best be addressed by professional such as social workers/nurses. Given the variable needs of each individual and his family, a continuous dialogue between the different care-givers is essential in finding an optimal balance between the medical versus non-medical needs. Finally, the success of a multidisciplinary approach also depends on the implementation of state-of-the art methodologies and technologies and often is a fertile ground for further research. As such, the multidisciplinary teams are also a source of up-to-date information for patient organisations.

EWS02. A multidisciplinary out-patient service for Huntington's disease.

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We describe an innovative multidisciplinary management clinic for Huntington's disease (HD) in Northwest England, serving more than 250 patients since it was established in 1990. Patients are seen annually for a comprehensive assessment including neurological, behavioural and neuropsychological examinations and a review of their medical treatment and social care provision. Although genetic counselling is outside the remit of the management clinic, close links with the Regional Genetic Service ensure that this is offered to at-risk family members where appropriate.
There is currently no treatment to arrest or delay progression of the disease, and medical care has traditionally been limited to making the diagnosis and treating involuntary movements. However, recent advances have established that patients can also benefit from symptomatic treatment for behavioural symptoms such as depression, irritability and obsessive-compulsive behaviours, while modern nutritional management using oral supplements or enteral feeding by gastrostomy tubes have reduced the incidence of life-threatening complications such as chest infections. These treatments are provided by a wide range of specialists including neurologists, psychiatrists, physiotherapists, speech therapists, dieticians and gastroenterologists, while social workers also play an important part in the support and management of affected families. Inevitably many professionals are unaware of the contribution which colleagues from other specialties could make. Regular review in the clinic assists coordination between disciplines, allows prompt identification and treatment of problems, and ensures that patients do not slip through the net, while the service also provides a valuable resource for clinical research and has been a model for similar developments elsewhere.

EWS03. A Multidisciplinary Approach to von Hippel Lindau Disease

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Von Hippel Lindau Disease (VHL) is an autosomal dominant disorder, which predisposes individuals to tumours of the brain, spinal cord, inner ear, eyes, kidneys, adrenal glands and cysts of the kidney and pancreas. Current guidelines recommend that individuals with VHL or

at risk of developing the disease should be offered regular screening tests. In order to coordinate screening and improve patient care a one-stop multidisciplinary screening clinic was established at Guy's Hospital in January 1998. Disciplines represented at each clinic are ultrasound, renal medicine, ophthalmology, genetics and neurology. The process of establishing and maintaining such a clinic and its benefits for patients and clinicians will be discussed.
A recent UK study has evaluated and compared the patient's perceptions of care across the two types of screening service provision for VHL disease - *One-Stop* services which offer the majority of screening tests for VHL on the same day and *Ad-Hoc* services where the patient attends separate appointments for screening tests over a variable timescale. Patients from eight centres across the UK were sent a postal questionnaire (response rate 61.5% (72/117) to elicit their views on

- their experience of VHL and screening,
- their perceptions of care using a multi-item scale addressing the co-ordination of their care e.g. access to care, timeliness of care.

The results of the study are being analysed in Spring 2004 and will also be presented.

EWS05. Information leaflets: Aiding patient understanding.

***T. J. Bussoli**;*

Guy's Hospital, London, United Kingdom.

Patients can be given a large amount of information during a typical clinical genetics appointment. To aid recall of such discussions, clinicians usually write to patients, following their appointments, to summarise some of the issues that were discussed.
We felt that there was a need to improve the presentation and quality of the information that we provide for our patients.
As a team, we worked together with a graphic designer to produce 50 different patient information leaflets, covering a wide range of topics in all areas of genetics. Much of the information is in written form but many leaflets include images of different concepts in genetics that we hope will aid patient understanding.
This talk describes how we approached this project, shows some of the results of our work and raises questions about the usefulness of such information.

EWS06. Seeing Chromosomes - translating genetic information into British Sign Language

***R. A. Belk**[1], A. Middleton[2];*

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Information can be made easily accessible to patients through the use of well-designed leaflets and, when used in conjunction with clinic letters, can have the additional benefit of reducing the workload of both clinicians and administrative staff. Both the North-West and East Anglian Genetic Services have adapted for their own use the excellent leaflets put together by the Guy's Hospital team in London. However, equality of access to this information for non-English speaking groups can only be achieved through accurate translation into minority languages.
Deaf people with British Sign Language (BSL) as their first language are one such group as the average literacy age in written English is 7.5 years. BSL cannot be accurately represented on paper. This presentation therefore considers the issues involved in planning leaflet translation onto video/CD-ROM. This is a starting point for investigating the accuracy of translation of genetic information, a particularly complicated subject. The translation of these leaflets back into written English will highlight specific terms and meanings that are particularly difficult to translate.
This information will be used to inform the planning of teaching for BSL interpreters who work within genetics. It is envisaged that teaching should include basic genetic concepts so helping interpreters to define and set in context terms such as 'genetic', 'gene' and 'chromosome' for their clients - BSL uses one handshape for all three of these terms so making explanation of each even more important.

EWS07. Translating genetics leaflets into languages other than English: lessons from an assessment of Urdu materials

***A. Shaw**[1], M. Ahmed[2];*

[1]Brunel University, London, United Kingdom, [2]Department of Clinical Genetics, St James University Hospital Leeds, United Kingdom.

Genetic counselors frequently counsel clients whose first language is not English, relying on interpreters and on supplementary translated written material. Here we highlight factors that counselors need to consider before using or commissioning translated leaflets. Our work is based on an assessment of leaflets translated into Urdu, the national language of Pakistan and available through U.K. genetics centres for use with Pakistani-origin clients. The two authors, who know Urdu as their second and first languages respectively, independently read each leaflet, checking for accuracy of information, ease of reading and understanding, cultural sensitivity, and contact details for Urdu speaking professionals. We found factual errors and confusing or very difficult text in all leaflets; some leaflets also contained culturally insensitive messages that could alienate users of genetics services. We consider the reasons for these pitfalls, and make recommendations to guide the future production of translated genetics leaflets.

EWS08. Cognitive theory and its utility in genetic counselling

***R. MacLeod**[1], A. Silver[2];*

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A workshop is planned to demonstrate cognitive techniques deriving from Cognitive Behavioural Therapy (CBT). We propose to briefly outline the model and explore why this model may be useful to genetic counsellors. Case vignettes will be used to highlight strategies for dealing with two frequently encountered problems in genetic counselling:
Handling uncertainty
Feelings of Guilt
While some patients experience strong feelings of guilt, we may falter as professionals in knowing how to approach the subject (Chapple et al., 1995). This difficulty may partly lie in our own counter-transference responses (Kessler, 1992). The recurring theme of uncertainty in genetic practice also presents a key challenge for professionals (van-Zuuren et al., 1997; Smith et al., 2000). We will draw on cognitive theory to help explain people's reactions and adaptations. Participants in the workshop will be guided to look at two cases reflecting the two themes of uncertainty and guilt. Cognitive Behavioural Theory will be used to consider what the counselee's underlying thoughts/beliefs might be in each case. Participants will be encouraged to think about the style of questioning that could help elicit important thoughts or beliefs.
The second part of the workshop will consider how these same cognitive techniques can be used to help deal with difficult thoughts or feelings that emerge as a consequence of our work. This relates not only to clinical competence, but also reflecting on the extent to which our own needs for support are met.

EWS09. A workshop on the use of non-verbal techniques in psycho-educational group work in genetic counselling

***T. Brouwer**[1], H. van Spijker[1], A. Blom[2], D. Rozendal[3];*

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Psycho-educational group work is a useful source of support in addition to face-to-face counselling in the different fields of medical genetics, like prenatal diagnosis, oncogenetics, neurogenetics, and cardiogenetics. It enables participants to share their emotions with people in the same situation and to clarify their own decision-making process. Moreover, it facilitates the process of coming to terms with the diagnosis and course of the disease. The presence of a professional group worker contributes to a well-balanced programme: from ensuring a free exchange of experiences and opinions, to providing a more structured approach for sensitive topics.

Social workers in clinical genetics in the Netherlands organise psycho-educational groups for hereditary breast and ovarian cancer (women at risk and patients), for Huntington's disease (separate groups for gene carriers, patients, and partners of patients) and for couples after termination of pregnancy for genetic reasons. In addition to the verbal exchange of experiences, information and emotions, non-verbal techniques are used to encourage participants to deal with emotional subjects. Using this specially developed material, it is possible to reveal the behavioural, cognitive and emotional factors and make them tangible in concrete situations. Similar techniques can also be used in individual counselling.
In the workshop we will discuss and explain the techniques and merits of working with non-verbal exercises and different kinds of material in various types of groups. There will be an opportunity for you to practice these techniques and we will use the family coat-of-arms as a model.

EP01. Health behavior and psychosocial reactions to genetic counseling among women with hereditary breast- and /or ovarian cancer risk.

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Background Genetic counseling is a offered to all Danish women at risk of developing hereditary breast- and /or ovarian cancer. The counseling is offered free of charge by the public health system after referral. It consists of counseling, pedigree drawing and verifying, risk assessment and, if possible, a genetic test. An increasing number of women are seeking counseling, however, the knowledge about the psychosocial consequences of this prevention strategy is still limited.
Purpose To investigate the consequences of genetic counseling in the following 3 areas:

- ∇ Health-related quality of life, breast cancer anxiety, general anxiety and depression
- ∇ General health-related behavior
- ∇ Breast cancer risk perception

Method A population-based prospective cohort study based on 400 women attending genetic counseling for hereditary breast- and /or ovarian cancer risk and two reference groups.
Data from the primary study population are collected by self-administered standardized, mailed questionnaires prior to counseling and at 14 days, 6 and 12 months post-counseling.
The reference groups consist of 400 women attending breast cancer screening, and 1500 randomly selected women from the Danish population. Data from the reference groups are collected by similar questionnaires, but only twice with one year apart.
Data from the doctors providing the genetic counseling are collected by questionnaires right after the counseling session.
Results Preliminary baseline and 2 weeks follow-up results will be presented. The perspectives for the further results will be discussed.

EP02. Women Discourse facing Prenatal Diagnosis

A. S. N. Marinho[1,2], J. C. LLerena Junior[1];

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A diagnosis of greater risk for diseases such as Down Syndrome acts as if most pregnant women already carry an affected child. This study aimed to investigate interactions and attitudes of women facing prenatal diagnosis tests and comprehended 1,400 hours of participation observation during medical appointment (1999-2002). Two critical conditions were assumed considering the psychological viewpoint: the pregnancy and the allegedly at risk disease in the child. The outpatient clinic assists in average 200 women every year; forty eight percent (48%) of those women were referred because maternal age factor. Fifty percent (50%) of the prenatal invasive exams indicated were not accomplished. The cultural background among patients was a decisive factor in comprehending and adhesion towards the prenatal exams. Furthermore, personality characteristics and emotional background were all important factors influencing most decisions and strategies toward genetic risk situations. The prenatal diagnoses process effectiveness does not dwell in the cure of the defective genes, but in the possibility of most women in producing a new narrative of different meanings about the risk situations. Most tensions created from the increasing gap between lay knowledge and medical technological attitude associated to the absence medical resolution once facing a catastrophic outcome to the baby (Brazilian law prohibits abortion, including for those with major congenital malformations) should be considered with care before submitting most women to undesirable and unexpected emotional feelings.

EP03. Attitudes towards predictive genetic testing for Alzheimer´s disease

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Objective Neuropsychiatric diseases are caused by the interaction of various susceptibility genes and environmental factors. As the contribution of each gene mutation is very small, the knowledge of single vulnerability genes will have little power to predict onset and progression of these diseases. The general understanding regarding the interaction of different genetic and environmental factors is still limited: Although the term „hereditability" is not anymore associated with Mendelian laws only, the understanding of complex genetic mechanisms is not yet widespread. To ensure that the results of psychiatric-genetic research are handled in a responsible way, their integration in a realistic context is essential.
Methods A representative sample of the German general population was interviewed about attitudes towards genetic tests for neuropsychiatric illnesses on the one hand and perception and interpretation of probabilistic risk information on the other hand.
Results & Conclusion Our results indicate that the majority is in favor of predictive genetic testing for neuropsychiatric diseases in general. Arguments supporting genetic testing were: „Prevention of the disease", and „possibility to prepare for the disease". At the same time however, most of the respondents refuse psychiatric genetic testing for themselves due to the following arguments: „psychological burden" and „lack of therapeutic possibilities". Furthermore our results show that the majority had problems in understanding probabilistic risk information. As the decision of an individual for or against predictive testing will mainly relay on his understanding of risk stratification, the ability to handle risk information will be of central importance in the future.

EP04. Risk perception and risk recall in genetic counseling for cancer

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The aim was to study risk perception and risk recall in the course of genetic counseling for cancer. The subjects were 46 Finnish clients of the genetic counseling for cancer, who also were notified of their risk level (not increased risk, moderately increased, markedly increased risk) by a genetic counselor. Perceived risk was measured as lifetime risk; that of a Finn of same age and gender; and risk based on family history. Also recalled risk was measured. At pre-counseling 7 of the subjects estimated their lifetime cancer risk low, 25 moderate, and 14 high. The figures were exactly the same for the perceived risk based on family history. In the counseling 5 clients got low, 21 moderate, and 20 high-risk notification. However, two weeks post-counseling, 4 clients recalled having received low, 35 moderate, and 7 high risk estimate. At 12 weeks post-counseling, the corresponding figures were 6, 33 and 7. In the discriminant function analysis, family history of cancer, optimism and social support were predictive of accurate risk recall, and at 12 weeks also better knowledge of genetics and cancer, and higher social status. Higher number of children was predictive of over-estimation. In general, there was a tendency to recall the notified risk in an optimistic way over time. Factors like familiarity with cancer, knowledge of cancer and genetics, and social support as enhancing accurate risk recall are amenable for interventions, also as a part of the counseling process.

EP05. A pilot study of a communication aid to facilitate risk communication with women from high risk breast cancer families

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Background: The literature on risk perception in women from high-risk breast cancer families reveals persistent over-estimation of risk, even after counselling. In this study, a Communication Aid was designed to facilitate discussion of risk between geneticists/ genetic counsellors and women from this high-risk population. **Method:** *Stage 1*. The Aid was developed by a team of experts. It was guided by the international literature on risk communication and a large multi-centre Australian study of risk communication. The 13 page full-colour Communication Aid used varying formats of words, numbers, graphs and pie-charts. It addressed a) the woman's subjective risk; b) the population risk of breast cancer; c) inherited breast cancer; d) the cumulative risk for women with BRCA1 and BRCA2 mutations; e) family risk factors; f) the woman's suitability for genetic testing; h) screening and management recommendations, and i) a re-assessment of the women's subjective risk. *Stage 2:* A pilot study of 27 unaffected women in four Australian familial cancer clinics was undertaken. Baseline and follow-up questionnaires were administered. **Results:** Compared to patient outcomes in a study undertaken in a similar Australian population in 2001, the use of the risk communication aid appears to be beneficial; more of women's expectations were met, breast cancer genetics knowledge was higher and risk perceptions were more accurate in the cohort receiving the aid. An analysis of change scores for anxiety and depression between the Pilot Study and the Comparison Study showed no difference in anxiety but a smaller decrease in depression in the Comparison Sample (p=0.06).

EP06. Culture and genetic services: a meeting of minds

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We report here on an initiative designed to further our understanding of issues of culture in the context of genetic services. A one-day meeting was convened whose purpose was to explore assumptions about culture and genetic services and to identify appropriate questions for future research. The definition of 'culture' for these purposes was a wide one including, for example, deaf culture. It was hoped that this approach would help participants to separate out issues to do with language and ethnicity from those to do with cultural beliefs and attitudes. Those invited to the meeting were chosen for their professional involvement with the topic either as a practitioner or as a researcher (or both). There were 28 participants. The day started with presentations from four speakers, following which participants identified questions to address in more detail in smaller groups. Groups were encouraged to identify what was known, and what needed to be known for progress to be made. All discussions were tape-recorded and treated as expert focus group material. Themes which emerged included: variations in practice between different genetics centres; clients' expectations and receptivity to the information being offered; the absence of agreed performance indicators; educational level as a sub-culture; conveying information as a core activity of genetic counselling; the need for a common language, both literally and metaphorically; the use of interpreters; information and the family. A number of corresponding questions for future research were identified.

EP07. Genetic Counselling for Cancer Susceptibility in Barcelona, Spain. Five years of experience.

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Familial cancer clinics are a recent development in Spain and little is known about the characteristics of patients who attend. Our Genetic Counselling Unit at the Catalonian Institute of Oncology in Barcelona, Spain, was created in 1999. So far, 2.660 individuals from 1.546 unrelated families with personal or familial history of colorectal (44%) or breast/ovarian cancer (42%) have been attended. A genetic test has been indicated in 511 families (33%). Sixty six percent of patients were referred by a medical professional, 13% by a family member and 21% stated that the referral had been initiated by themselves. Most of the patients primarily wanted information about their personal risk (46%) or their siblings risk (43%). Nineteen percent wanted to reduce their level of anxiety and only 5% wanted to plan their future. Twenty-six percent of the interviewed patients did not know about cancer genes. When asking about the reasons to accept a genetic analysis, the majority of the patients would accept it to help the family, principally siblings, and to improve knowledge about their personal risk and how to handle it. Only 10% of the patients wanted information about prophylactic methods. Fifty six percent of women considered prophylactic mastectomy as an exaggerated procedure, 20 % have never though about it, and only thirteen percent will consider it if they carried a BRCA gene mutation. In conclusion, we try to take into consideration the informal and psychological needs and concerns of our population in order to best tailor the cancer genetic counselling.

EP08. Clinical Service and Consumer Group: Strength in Partnership

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Support and advocacy organisations specifically for people affected by genetic conditions exist in many countries, and in Australia in three states. In Australasia, the model of a consumer group arising from a partnership between clinical service providers and consumers themselves, and co-existing in the same physical location as the clinical service, is unique. This paper investigates the features of such a model and explores the advantages and disadvantages, both perceived and actual. The paper elucidates the role of the service provider in the setting up stages and the ongoing existence of the consumer group. Highlighted are the achievements to date which have been made possible by the partnership, including:

- The delivery of a professional counselling service that included the support groups in a „continuum of care";
- The high rate of referrals by genetics specialists to support groups;
- The successful set-up of new groups for very rare conditions;
- The establishment of mechanisms for meaningful consumer input into planning clinical genetics services.

The future plans of the consumer organisation include setting up more groups for rare conditions and the ongoing input of consumers into planning services.

EP09. Supporting women after abnormal findings during pregnancy in cases where the pregnancy is continued

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Modern methods of prenatal diagnostics leave an increasing number of women faced with an abnormal finding that might raise the question of terminating the pregnancy. Women and couples (still) considering whether to continue or terminate the pregnancy should undergo broad and possibly multiprofessional counseling and seek support that enables them to reach a sustainable decision.
Models for support during the process of a late-term termination and the time after have been developed and tested during recent years. In contrast, women and couples who decide to carry the child to term experience a lack of professional support, particularly when the

prognosis for the child's survival is questionable.
The work presented here intends to show ways of dealing with a pathological finding by initiating multiprofessional counseling and support that also works in cases when the pregnancy is continued. Support is adjusted to the needs of the pregnant woman and her partner and is modified with respect to certainty of diagnosis and the type of prognosis given. Special attention is devoted to pointing out the interactions and synergies within a multiprofessional cooperation.

EP10. Improving services for patients with hearing loss or visual impairment

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Genetic counselling for people with visual impairment and/or hearing loss requires specialist communication support. Following a successful development bid, two half-time genetic counsellors were appointed to improve the service for this group. This presentation details some of the implemented and proposed changes. Many of these are simple adjustments whilst others are longer term.
People seen with hearing loss fall into three groups: hearing families with deaf children, congenitally deaf (Deaf) adults and people with acquired hearing loss, most commonly NF2. Each of these groups has different communication needs and attitudes to their hearing loss. Amongst other projects, a dedicated deafness clinic has been established, a 'communication sheet' is now used to help with planning and recording of support, liaison with regional audiologists is improving evaluation of children's hearing loss prior to referral, translation of selected leaflets into British Sign Language is planned and a training day for interpreters working in genetics is under discussion.
For those with visual impairment, genetic counselling input has been introduced in three additional multi-disciplinary ophthalmic/genetic clinics each month to improve the quality and effectiveness of our service. Communication improvements include using RNIB clear print guidelines for letters, leaflets and other printed materials and the provision of information in alternative formats including audiotape, computer disc, Braille and the use of tactile diagrams. Establishing links with professionals and lay organisations is enhancing follow-up support.

EP11. Psychosocial Impact of Predictive Testing in Hereditary Ataxia type 2.

***M. Paneque Herrera**;*

IBMC., Porto, Portugal.

The Predictive Test Program represents a novel experience in Cuba applied to a hereditary diseases with a dynamic mutation and with a dominant autosomic inheritance pattern. Similar precedents were stated in the specific case of Huntington Disease , the first genetic affection that relied on molecular markers for its predictive diagnosis, thus becoming a paradigm of such type of study.
This work values the main differences and contact points, as well as those psychosocial factors that particularize the different contexts in which both programs take place, in order to contribute with the development in Cuba of presymptomatic diagnoses of hereditary diseases of late appearance.
It is necessary to point out that in the generalization and analysis of the results of this comparative study there are limitations since non homologous experiences are analyzed as for the disease, the extension, the number of participants, obviously higher for the HD and the social contexts in which they take place. However, as the program designed for the SCA2 took the one that was previously established for HD as a model, it was considered appropriate to determine those aspects of interest or points of conflict described in the vast international experience for this illness, that could contribute to the development, investigation and evaluation of the predictive tests for Cuban Ataxia

EP12. Big Country - Small Population. Models of Service Delivery from an Australian Perspective

***C. L. James**, K. Crowe;*

Royal Children's Hospital, Herston, QLD, Australia.

The development of Genetic Counselling services in Australia has been heavily influenced by the geographic distribution of the population. The majority of the population is concentrated in coastal areas where there are only a few major cites with metropolitan sprawl. The remainder is dispersed across thousands of kilometres of varied terrain, which includes coast, rainforest and harsh arid land. These geographical barriers have led to the development of three main types of service delivery: - Centralised with permanent outreach positions, centralised with visiting outreach services and dispersed stand-alone services. Each state has developed only one type of service delivery in response to the distribution of established health services and varying population density. The benefits and drawbacks for these three types of service will be explored for both the service providers and clients.
The main service providers are genetic counsellors and geneticists. Genetic services within states can vary with major units in cities, metropolitan outreach and rural outreach. Issues such as professional isolation, supervision and education opportunities, the use of technology such as video-conferencing to supplement or replace existing services will be examined.
Issues for the clients can include cultural differences between metropolitan and rural areas, confidentiality of information, service providers familiar with local services and supports, transport and access to services.
These tried and tested methods of service delivery can serve as models for other countries and areas with similar population distribution or obstacles to equitable access to service such as transport issues and geographic isolation.

EP13. Impact of Turner syndrome (TS) on the psychosocial development of 25 Portuguese patients

***A. Sá Leonardo**[1], A. Sousa[1], A. Medeira[1], I. Cordeiro[1], M. Ávila[1], J. Gonçalves[2], M. C. Santos[1], H. G. Santos[1];*

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Introduction: TS consists of short stature, short and broad neck, hearing problems, primary amenorrhea and sterility, affecting one in every 2500 female newborns. Some authors have described a characteristic neurocognitive profile as well as problems in psychosocial development, but no data exists for the Portuguese population.
Objective: To study the impact of TS on the quality of life of 25 patients (aged 2 to 57).
Methods: A clinical interview was conducted. Specific tests were applied to evaluate cognition, personnality and self-concept; behaviour scales were also used.
Results: All patients were active members of the community. Two girls with moderately impaired cognition required special education; 7 other patients went on for a college degree. There was a direct correlation between hearing disabilities and learning problems. Most patients had decreased arithmetic, perceptual and visual-motor skills. Three in 16 adult TS patients recorded Neuroticism levels below average, contradicting other studies which reported a high tolerance for stress. Forty-percent of all adult patients had some psychopathology history and 1 among the 4 children had attention deficit hiperactivity disorder (ADHD). Young and adult patients revealed average self-concept, however peer relations were poor and there was significant emotional dependence on the family. Short stature and sterility were the features with most impact on the quality of life of these patients.
Conclusion: Portuguese TS patients appear to have the necessary flexibility to participate in community life. This research has yielded important data for Genetic Counselling in Portugal and resulted in the creation of a TS patient association.

EP14. Professional responses to nondisclosure of genetic information

***E. A. France**, A. Clarke;*

Institute of Medical Genetics, Wales, United Kingdom.

The patient's right to privacy, and the professional's obligation to respect their confidentiality, are both challenged by the argument that genetic information is shared, belonging to a family rather than the individual. If important genetic information is not passed on, then relatives could sometimes be at avoidable risk of disease complications. Professionals are concerned that they may be legally held liable when such complications arise, and are beginning to alter the wording of consent forms for genetic testing to coerce those being tested to make their results available to others.
The arguments used to justify force disclosure or coerced consent to disclosure often fail to take account of the temporal dimension or the medical uncertainty about the potential for avoidable harm form non-disclosure. We present three cancer genetic counselling cases where non disclosure was recognised as an issue, but supportive contact was maintained with the client and - after some years- they informed their relatives about their genetic status. The benefits to the clients and their family relationships from allowing them time for such decisions are very substantial and are easily omitted form bioethical discussions of the potential harm of non-disclosure.

EP15. Faciliating family communication about predictive genetic testing for HNPCC: the proband's perception.

***C. L. Gaff**;*

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Although it is known that genetic information is rarely completely shared in families, genetic professionals rely on family members to notify relatives that predictive testing for cancer predisposition is available. This task usually falls to the proband - the affected individual in whom the causative mutation is first found. Probands can find this a burden and professional support during this process has been recommended (Bonadona et al, Cancer Epidemiol Biomarkers Prev 11: 97-104).
With the goal of enhancing support for probands and maximising information dissemination, we explored the utility of genetic counselling and communication aids to probands informing relatives that predictive testing was available for Hereditary Non Polyposis Colorectal Cancer (HNPCC).
Five men and seven women were interviewed by phone.
Respondents reported few difficulties in communicating information about testing. Nonetheless, all respondents failed to inform some biologically, socially or geographically distant relatives. Generally, respondents would not change the way they informed family members, although it appears that men may require more guidance and support during this process. Probands conceded that aids such as letters would be helpful for *other* people who had communication problems, but were seen as a source of accurate information rather than a way of informing additional relatives.
Respondents found communication relatively easy and probably represent the 'best case' scenario. Genetic services should consider a more active role, working in partnership with the proband to ensure that all at-risk family members have access to predictive genetic testing.

EP16. Exploring families' psychosocial needs after genetic diagnosis: Perceptions of genetic counselors.

***D. R. Schild**;*

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A new genetic diagnosis is likely to raise significant psychosocial reactions in a family. There is a body of literature on the psychological impacts of these diagnoses, but only anecdotal evidence about other psychosocial disruptions. In addition, because, genetic counselors in the United States do not usually have long-term contact with their clients, there is little know about the long-term psychosocial needs of families with a new diagnosis. I report here on the first of two pilot studies of families' psychosocial needs. The first study focuses on what genetic counselors understand about families short-term needs. The second study will be based on a patient and family sample and will expressly include long-term adjustments.
We explored the knowledge and practices regarding psychosocial issues with the primary provider of genetic services, genetic counselors. We interviewed 20 genetic counselors about the psychosocial issues dealt with in counseling sessions, what services exist, and client barriers to using the services. Genetic counselors identified a complex set of needs for psychological, support and concrete services. Most counselors address likely anticipate and discuss psychological responses and family social support with patients. Few discuss such other psychosocial issues as coping strategies, environmental resources, and changes in family roles. Counselors discussed barriers to comprehensive psychosocial services, and most often cited their lack of training, limited time with clients, and external barriers, most importantly a lack of insurance coverage for care. Counselors acknowledge that many families need long-term follow-up care, and often refer patients to social workers for those services.

EP17. Rehabilitation treatment in HD: benefits beyond motor and verbal improvement.

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Huntington's disease (HD) is a neuro-degenerative, autosomal dominant, late-onset disease characterized by slowly progressive movement disorders, cognitive deterioration and psychiatric manifestations.
In 1999 we started a rehabilitation protocol for HD patients at the Caring Home «Nova Salus» of Trasacco, in Abruzzo Region. The interdisciplinary treatments were performed at an intensive regimen for 8 hours a day per 6 ½ days (Saturday only in the morning, Sunday free), for 3 weeks for a maximum number of 3 admissions per year. The effect of the rehabilitation was evaluated both in terms of motor performance, quantitatively assessed through motor scales, and in terms of subjective evaluation by patients and caregivers. Here we report the results obtained through the latter approach.
An ad hoc Questionnaire was devised and sent to 59 subjects. Forty-five of them (76%) sent back the filled in questionnaire.
Overall positive effects of the rehabilitation experience were reported by 97.6% of respondents. Improvements were reported for body control (89.7%), speech (85.3%), balance (81.3%), gait (80.9%) and swallowing (80.9%). Positive effects were also reported for several psychosocial aspects, namely mood state (89.4%), establishing new friendships (88.8%), reducing apathy (81,3%), family relationship (78%) and social relationship (74.4%).
The positive, although temporary, effects obtained with the treatment will constitute the base for developing education programs for health care providers in our country.

EP18. Diagnosis of a non-life-threatening, progressive, neuromuscular condition. ,The impact on life'.

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Receiving a diagnosis for a progressive, neuromuscular condition such as Charcot-Marie-Tooth disease (CMT) or Myotonic muscular dystrophy can be news of major gravity. Initial symptoms of such conditions are often rationalised through activities of daily life, accepted as 'personal traits' or simply misdiagnosed for many years. A correct diagnosis is often not obtained until well into adulthood, as symptoms become more pronounced with age. This revelation can have numerous implications for an individual and their whole family. However literature on impact of diagnosis in progressive, non-life threatening conditions is scarce.
This study collated data from 25 questionnaires. A series of open questions explored the impact on life following diagnosis of CMT or myotonic dystrophy. An 'Impact of Event' scale provided a quantified measure of the degree of diagnostic impact. 6 in-depth interviews gave greater insight into personal experiences of diagnosis.
As initial shock subsides post-diagnosis, a need for more information

is evident as individuals strive to deal with considerable uncertainty, disruption to self-identity and changes in family dynamics.
This research provides a greater insight into the positive and negative outcomes that can arise following a diagnosis of CMT or myotonic dystrophy. Awareness of key factors influencing degree of diagnostic impact will be beneficial to practice, allowing better recognition of client needs and development of techniques for facilitating acceptance of the diagnosis.

EP19. The impact of having relatives affected with breast cancer on psychological distress in women at increased risk for breast cancer

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Background: Women at increased risk of developing breast cancer (BC) may report elevated levels of distress because of the previous and sometimes recurrent confrontation with BC in their family.
Objective: To determine the association between psychological distress and the degree of confrontation with BC in a relative.
Material and Methods: Participants adhering to a regular surveillance program currently under study in the Netherlands (MRISC-study) filled in a questionnaire two months prior to a surveillance appointment, comprising demographics, general and BC specific distress and several aspects of confrontation with BC in the family.
Results: 347 out of 351 participants (mean age 40.5 years) had at least one relative affected with BC. The following variables were significantly, positively related to BC specific distress: having at least one affected sister ($n = 105$; $p < 0.04$); close involvement in a sister's BC process ($n = 94$; $p < 0.03$); less than three years since the BC diagnosis in the relative ($n = 114$; $p < 0.05$), especially in a sister ($n=30$; $p < 0.03$). General distress did not show any significant associations with the confrontation of BC in the family.
Conclusion: BC specific distress is positively associated with the confrontation of BC in a sister, and with a BC diagnosis in a relative that was less than three years ago. These findings underscore the impact of having to deal with BC in the family and have to be considered in the counselling process.

EP20. Telemedicine for hemophilia: keeping in touch

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Quality of life of hemophiliac patients, already benefiting from continuous prophylaxis, can be further improved with patient monitoring by telemedicine. Today, parents of a hemophiliac child are afraid of not being able to evaluate the conditions of their child facing traumatic events and therefore of underestimating them. At the same time, if the family lives far from specialized centres, parents also fear that episodes are ill treated by not specifically competent staff.
In order to help families with newly born hemophiliac children to be autonomous without feeling abandoned, we started a project of telemedicine which allows routine and emergency visits to be performed avoiding patients' transfer and thus saving time through a continuous multimedia connection between patients' homes and health care centres.
Four patients aged 24 to 60 months with moderate-to-severe hemophilia A and B were enrolled in the study. Their families were provided with a multimedia workstation (PC, Webcam and printer) with ADSL connection to the health care centre. Duration of the project is one year, including routine monthly visits and round-the-clock medical and nursing emergency care through Webcam visual visits, while parents are trained to perform tactile evaluations.
Effectiveness of the project will be evaluated through the analysis of STAI Y 1 and 2 and Zung-DEP scales administered to parents on patient enrolment, during routine and emergency visits, and at the end of the study period, and through a self-evaluation questionnaire administered at the beginning and at the end of the project.
Supported by Bayer Health Care

EP21. Attachment in families with Huntington's disease: a paradigm for clinical genetics

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This study investigated adult attachment representations in 32 adults at 50 % risk for Huntington's disease (HD) who were raised by an affected parent.
Based on daily interactions, children form mental representations of the relationship with their parents. A child is securely attached if he seeks proximity to his parents when frightened, tired or ill. He can also be attached in a dismissing (insecure-avoidant) or preoccupied (insecure-resistant) way. In addition, indications of unresolved trauma and loss can be determined.
Attachment theory assumes that attachment experiences lead to a working model that will be used in social relationships throughout life. This working model is assessed in adults by means of the Adult Attachment Interview[1].
We found a lower percentage of secure attachment representations, a higher percentage of preoccupied representations, and a higher percentage of unresolved/disorganized representations, compared with the non-clinical population.
A relatively late start of the parent's HD-career was found to be associated with a secure adult attachment representation. Death of the HD-parent before the child's 18th birthday was associated with an unresolved/disorganized adult attachment representation.
This study also investigated the relationship between adult attachment representation and psychological well-being of people who were raised by a parent with HD. We found the unresolved/disorganized group to be more anxious.
We will present three cases in some detail to highlight possible pathways from childhood experiences concerning HD to adult attachment representation.
[1] George, C., Kaplan, N., & Main, M. (1996). Adult Attachment Interview 3rd edn. Department of Psychology: University of California, Berkeley.

EP22. The impact and outcome of genetic referrals of children with idiopathic developmental delay from the parents' perspectives.

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This study aimed to explore the impact and outcome of genetic referrals (and possible genetic diagnosis) of children with idiopathic developmental delay from the parents' perspectives.
A qualitative research method was employed, using semi-structured interviews. The sample consisted of fifteen participants - thirteen parents and two grandparents of children with idiopathic developmental delay.
The main findings include:- parents' concerns are not always acknowledged; parents experience difficulty and delay in obtaining a genetic referral for their child; parents want certainty; insufficient information is given to parents; parents cope better with more information - even in the absence of a diagnosis; and lastly there is a clear benefit to referring such children to genetics.
Recommendations are proposed for both health professionals and genetics services. Those for health professionals include: acknowledging parents' concerns about their child; making prompt referral to genetics where required; raising their awareness of the psychosocial impact of such genetic referrals (and/or genetic testing) on the parents which would improve handling and co-ordination of such referrals. Recommendations for genetic services include: providing parents with sufficient, appropriate and understandable/"lay" information; and keeping parents informed throughout the referral and evaluation process.

EP23. Genetic Counseling and the Psycho-Social Implications in Several Sexualization Disorders

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The sexualization of the human body, a progressive process developed as a genetically initiated and conditioned program (at least 30 different genes) is not a vital phenomenon for the organism, but extremely important for the species survival. Organizing factors, gonadal steroids, peptide hormones and tissue receptors are also involved in sex determination. Interruption at each level of sexual organization will give rise to various degrees of disturbances in sexual development.
The sexualization disorders, a delicate problem for the genetically consulting, affect just the reproductive system and they are not usually associated with morbidity or with the decrease of the span of life.
There has been observed the psychological and social impact on the probants and their families. The patients have been distributed as a result of the genetic consultation in three categories: gonadal dysgenesis with mixt phenotype (true hermaphroditism-1case), male pseudohermaphroditism with anomalies of the target tissue dependent on androgens (complete and incomplete androgen insensitivity syndromes AIS-9 cases), and transsexuals - 1case.
This case was offered genetically consult and advice at the patient's own request. The orientation through the genetic consultation towards a certain sex was made after cytogenetic, hormonal and hystopathological investigations. One of the affected individuals with complete AIS has two relatives with the same disorder.
According to the presence or absence of the genital ambiguity, to the consulted patients' age, to their knowledge and mentality, there are some particularities for each sexualization disorders category, which are described in the present study.

EP24. Diagnosis of a Genetic Condition:an exploration of the impact on carrier grandparents.

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It is recognised that genetic counselling practice includes the identification of individuals who are at risk of being carriers of a genetic condition, following diagnosis in a family member. However, experience has shown that there is a reluctance to offer testing to the grandparents of affected individuals unless it is deemed necessary, in view of the potential impact that this may have on them. A literature review has identified that there is very limited research, which explores the impact that a positive carrier test has on grandparents. The aim of this small qualitative study was to identify any common themes pertaining to the feelings of grandparents who have had a positive carrier test within the last five years.
Six grandparents were interviewed and audio taped, and transcripts of the interviews were analysed to identify key themes. Results indicated that learning of a genetic condition in their grandchild has a considerable impact on the grandparents. A positive carrier test has had an immense impact on how the grandparents interviewed viewed themselves. They described strong feelings of guilt and responsibility, as well as a need to understand where the gene/chromosome change had originated from in previous generations. However, not one of the grandparents interviewed regretted having the test.

EP25. The impact of very early diagnosis in Huntington's Disease

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The diagnosis of Huntington's disease remains a clinical one, traditionally based on finding motor features of the condition. With the introduction of mutation analysis, diagnostic uncertainty can now be rapidly dispelled. This could encourage earlier diagnosis where in the past more hard evidence might have been sought to avoid a false positive diagnosis. Although the diagnosis may still be accurate, clinicians may have more confidence in making it at a much earlier stage in the illness than previously.
Prior to the progressive illness many individuals display prodromal signs of HD, sometimes for long periods. These can include motor restlessness and sometimes occasional low amplitude choreiform movements. It is well recognised that increased emotional arousal can worsen the involuntary movements in sufferers. Predictive and diagnostic testing are nearly always very stressful for the testee. This could provoke physical signs which subsequently subside or even disappear. If there are accompanying cognitive changes of equivocal significance, and psychological changes like depression or irritable mood swings, non-specific but common findings in those with HD, the temptation to make a diagnosis may be further increased.
Two of the cases described are of men, both diagnosed in the very early stages of HD. One(33) gave up his job, stopped driving and adopted a sick role almost immediately The other(39)subsequently underwent, divorce but has remained stable, working, driving and coping well five years on. These cases suggest that early diagnosis in HD should be approached with caution and potential drawbacks considered carefully beforehand.

EP26. Does genetic counselling for disorders with variable expression resolve or create uncertainties, with respect to reproductive decision making?

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Genetic counselling focuses on supplying clients with definitive information with which to make decisions about reproduction and lifestyle. Variable expression, which is a phenomenon seen in TSC can complicate decision making as it makes the prediction of severity in future offspring impossible. This is a study of the views and experiences of 5 individuals at risk of having a child with TSC, undertaken to determine how individuals made reproductive decision is the light of variable expression.
Audiotaped semistructured interviews were conducted with participants to solicit their views in a number of areas of interest, including their knowledge of both the clinical and genetic aspects of TSC, their own experience of the condition and the reproductive choices available to them. Interview transcripts were analysed to identify common themes, which may have influenced participant's reproductive decision making.
Personal experience of the severity of some of the symptoms associated with TSC emerged as having a powerful influence on the decision making process. Individuals knowledge of the condition and the spectrum of severity that can be seen with TSC were varied in this group. Participants responses indicated that the greater the understanding of variable expression, the more definite individuals were about wanting to explore reproductive choices that would mean a child would not have the severe learning difficulties that can be associated with TSC.

EP27. Acceptance of novel diagnostic technologies for prenatal testing among parents of disabled children

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Prenatal diagnosis of chromosomal aberrations in most instances is followed by selective abortion, as no therapies are at hand.
This fact has given rise to the fear that congenitally handicapped persons may be seen as an evitable burden, with the consequence of eugenic tendencies in society. Consequently, „pro-life" activists took a fundamental stand against the development and application of prenatal testing.
The goal of our study was to assess the influence of having a handicapped child on parents' attitudes towards prenatal testing. 926 parents of normal children, children with Down syndrome, and

children with a mental retardation of unclear diagnosis were asked about their opinion considering present and future options of prenatal testing. Parents in general rejected the promotion of research aimed at the reduction of live-birth-rate of children with disabilities, and a considerable amount of people even opted to prohibit this kind of research. On the other hand, the major part would use novel, non-invasive technologies themselves, and about half recommended establishing them as standard procedure in pregnancy surveillance. About one third would even use pre-implantation diagnostics as a routine procedure, if they were allowed to. While the acceptance among parents of children with Down syndrome was high as well, they were considerably more sceptical about new technologies. These results show that on average, public knowledge about prenatal testing is fragmentary at best. Moreover, the acceptance of prenatal diagnostics is very high, as ordinary people often do not see the conjunction between prenatal testing and selective abortion.

EP28. Living with shadows: contextualizing the experience of being at-risk and reaching a decision about prenatal genetic testing

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As genetic tests enter clinical practice with increasing frequency, research is needed to explore the experiences of individuals who are living with genetic risk. Grounded theory (Glaser and Strauss, 1967; Strauss and Corbin, 1998) is one qualitative research method that allows for such experience-based inquiries. The intent of this particular study was to generate a substantive grounded theory that would portray insights into the experiences of women and couples who were offered prenatal genetic testing because of the mother's age and to draw from this theory implications for genetic counseling practice and research. Conversational interviews were used to elicit stories from 24 participants. Interpretation of the interview transcripts using an interpretive (as compared to a post-positivist) grounded theory method suggests that participants did not approach the decision to accept or reject prenatal testing as a medical decision but rather as an emotionally charged existential dilemma. The participants' responses to the angst associated with their experience of being at risk and making genetic-related decisions were portrayed through a narrative typology (e.g., submerging the angst, facing the angst with faith). The metaphor of shadows is used to convey the ways in which participants drew upon the temporal nature of human existence to make meaning of the existential experience of living with genetic risk. The central thesis of this theory argues for a genetic counseling stance that acknowledges the importance of being attuned in an "empathetic moment" to the variations of angst that genetic counseling clients might experience when confronting genetic-related decisions.

EP29. Different perceptions and attitudes regarding prenatal testing among service providers and consumers

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Background: It is well accepted that an appropriate prenatal care policy should reflect the needs and attitudes of consumers as well as opinions of service providers.
Objectives: To compare health professionals' and women's attitudes regarding necessity and extent of prenatal testing, and to compare these attitudes to women's actual behavior, in relation to cultural, sociodemographic and professional characteristics.
Methodology: The study was conducted in southern Israel. Jewish women (n=596) were interviewed by phone 5-8 weeks postpartum, using a structured questionnaire. Health professionals - gynecologists, pediatricians, neonatologists, geneticists and public health nurses (n=351) filled out a questionnaire, designed to parallel women's questionnaire.
Results: Health professionals were significantly more supportive of comprehensive prenatal testing than women (61.1% vs. 34.1%). Yet, professionals were aware of women's preference: 72.1% believed their patients preferred basic tests. For women, age over 35, Ashkenazi origin, and better knowledge regarding tests significantly predicted preferring comprehensive testing. Profession, gender (female) and secularism were significant predictors of that attitude among health providers. Women's use of prenatal tests (except for routine ultrasound scans) was lower than recommended by care providers, perhaps reflecting the differences in attitudes towards comprehensive testing. These differences are consistent with the findings that a given probability for Down's syndrome was considered high by more professionals than consumers (93.4% vs. 60.6%), and for all congenital abnormalities, more professionals than consumers justified pregnancy termination.
Conclusions: Providers and consumers of prenatal services differ in their perceptions and opinions. Policy makers should have mechanisms in place to properly represent this diversity.

EP30. Coping and emotional difficulties after termination of pregnancy (TOP)

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There is some controversy as to which coping styles are more effective preventing emotional difficulties after a loss, and specifically after a perinatal loss or termination of pregnancy (TOP). We have interviewed 81 women, 15 days after TOP, using the Moos Coping Responses Inventory (CRI), the Beck Depression Inventory (BDI) and the Zung State Anxiety Scale (SAS). Six months after termination we used BDI, SAS and Perinatal Grief Scale (PGS).
We found that (1) 'emotional discharge' (highest correlation for PGS, r=0.55 and p<0,01 for PGS) and 'problem solving' (for PGS, r=-0.41 and p<0.01) were correlated with perinatal grief, depression and anxiety; (2) 'approach coping' was negatively correlated with indicators of emotional problems, excluding anxiety on first evaluation (r=-0.39 and p<0.01 for PGS); (3) 'avoidance coping' had a positive correlation with anxiety and depression, only at the first evaluation (r=0.32 and p<0,01 for BDI); (4) 'positive reappraisal' had a negative correlation with all indicators, at both moments (r=-0.44 and p<0.05 for PGS). (5) Linear regression of all coping items (CRI), for each dependent variable, showed that some *itens* were predictors of emotional difficulties: 32, 34, 46, 11 and 29 for perinatal grief (R^2=0.66), *itens* 32 and 8 for depression (R^2=0.32), and 32 and 35 for anxiety (R^2=0,39).We conclude that individuals coping more actively with loss after TOP (those using problem solving coping), may have less depression, anxiety and symptoms of perinatal grief. On the other hand, those who try to discharge their emotions may have higher levels of emotional distress.

EP31. Layer upon layer of uncertainty: The complexities of carrier status determination in Duchenne muscular dystrophy

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Accurate determination of carrier status is the first step in rational reproductive decision making among female relatives of Duchenne muscular dystrophy patients. For women giving birth to the first male in their families with DMD, the situation can be complex and obscure. In a recent American survey, several findings challenge common assumptions.
The traditional estimate that two-thirds of cases occur in families without a previous history may require revision, as 88% of mothers of a son with Duchenne reported no known family history of the disorder. The earlier approximation may underestimate the rate of spontaneous mutation in the population, or indicate a reduction in the number of births to women positive for family history.
Misconceptions regarding the accuracy and limitations of carrier testing exist not only among women, but apparently among their physicians as well. Women reported being told that they are or are not carriers on the basis of CK testing alone. When the proband's mutation is not known, even DNA testing may mislead. Absence of common deletions and duplications may give a false sense of security when the possibility of point mutations is overlooked. If complete gene sequencing is negative, the possibility of germline mosaicism cannot be ruled out. Women differed in their awareness of

and response to such uncertainty. The absence of family history was strongly emphasized by many respondents. Whether a woman was identified as a carrier had implications for how she was perceived by self and partner.

EP32. Predictive BRCA1/2 genetic testing: Why do men do it?

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Purpose: Men who have a family history of breast and/or ovarian cancer may be offered a predictive genetic test to determine whether or not they carry the family specific *BRCA1/2* mutation. Male BRCA1/2 carriers may be at increased risk of breast/colon/prostate cancers. Relatively little is known about at-risk men's decision-making about BRCA1/2 testing. This ongoing qualitative study explores the influences on male patients' genetic test decisions and the impact of BRCA1/2 predictive genetic testing upon high-risk men and their immediate family.
Methodology: Twenty-nine in-depth interviews have been undertaken with both carrier and non-carrier men and immediate family members (17 male patients, 8 female partners 4 adult children), thus far. These explore: their experiences of cancer and genetic testing, decision-making about testing, family support, communication of test results within the family, risk perception and risk management
Findings: The influences on men's testing decisions, such as, altruism, anxiety about own health and own/partner's anxiety about children's risks or curiosity will be examined. Different types of patterns of family communication about DNA-testing and the test results will be described. Some methodological and ethical issues arising during the course of this study will be discussed.

EP33. Prophylactic surgery and surveillance practices in the year following predictive testing for hereditary breast/ovarian cancer.

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This is a report about uptake of breast/ovarian cancer screening and prophylactic surgery during the year following disclosure of the predictive test result for hereditary breast/ovarian cancer (HBOC). Seventy-one unaffected women who had a predictive test were eligible to be contacted for the 1-year follow-up assessment. Data were gathered by means of semi-structured interviews and self-report questionnaires in 68 participants (34 carriers/34 non-carriers).
Nine percent of carriers decided to have prophylactic mastectomy, 71% did not intend to have prophylactic mastectomy and 21% would consider it.
Regarding breast cancer screening of participants >29 years, we found that 64% of carriers had monthly (or more frequent) breast self-examination (BSE), 95% of carriers had semi-annual (or more frequent) clinical breast examination (CBE) and 95% of carriers had a mammogram. Uptake of CBE and mammography significantly increased from pre-to post-test ($p<.0001$; $p<.05$). Carriers had higher uptake of semi-annual (or more frequent) CBE than non-carriers (95% vs 30%, $p<.0001$). The proportion of carriers who had a mammogram was higher than the proportion of non-carriers (95% vs 60%, $p<.01$).
Of the carriers >34 years eligible for prophylactic salpingo-oophorectomy, 75% had prophylactic salpingo-oophorectomy, 13% did not want prophylactic surgery, 13% had concrete plans or would consider it in the future. All carriers >29 years who opted for regular surveillance of the ovaries, had ultrasound of the ovaries.
Our results demonstrate a significant impact of predictive testing for HBOC on health related behavior.

EP34. Preserving a moral space: the patients' perspective on the ethics of genetic testing

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The ethical issues surrounding genetic testing have been extensively explored by medical ethicists, practitioners and policy makers, as genetic testing becomes a standard part of medical care. From the point of view of the patient, however, the moral difficulty in genetic testing may be less to do with conventional ethical parameters such as the status of the embryo, or the risk/benefit balance to the individual. Psychological and social factors, including perceived control over one's life and balancing responsibilities to children and other family members, take a place in the ethical evaluation. The decision to have a genetic test (or not), is a process rather than an event. Moreover, for predictive testing in particular, deciding *when* to have a test may be as significant as the test decision itself.
In this paper we will refer to empirical data from an ongoing qualitative, interview- based study of the decision-making process in predictive genetic testing for cancer, Huntington's disease, and prenatally. We suggest that how the process is undergone is an important element in whether patients evaluate their choice as a morally satisfactory one, experience a sense of control, and integrate the genetic knowledge they are given into their lives. Interpreting what the decision-making processes mean in terms of the patient's ethical evaluation and sense of moral competence, we also draw out some important implications for genetic counselling, especially in terms of patient autonomy and the patient's sense of moral agency.

EP35. Psychological impact of a carrier result from pre-symptomatic testing for hereditary amyloid neuropathy (type 1 - Portuguese, Andrade)

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Hereditary amyloid neuropathy (HAN) - type 1 (Portuguese, Andrade) is a severe autosomal dominant neurodegernerative disorder with late onset, for which only a liver transplant may offer some hope of prolonging life. The aim of this study was to evaluate the psychological impact of a carrier result in the context of a pre-symptomatic testing.
We followed 70 individuals (44 females, 26 males; mean age 24.3 years), diagnosed as careers for the mutant gene for at least the first 6 months, in order to answer the question: how does this potentially harmful result affect them emotionally, in terms of depression and hopelessness?
The scores of depression and hopelessness, reached through the application of two scales (Beck Inventory of Depression, 1961; Beck Hopelessness Scale, 1974), were chosen as indicators of the psychological state of each subject, and taken at three moments: (1) before pre-symptomatic testing; (2) three weeks (depression scale) and (3) 6 months (hopelessness scale) after the disclosure of the results.
Our data showed: (1) the scores of depression and hopelessness did not achieve pathological levels, at any moment, for the majority of subjects; (2) and that awareness of a carrier result seems to not cause a negative psychological impact or affect the individuals concerning their hope to their future life.
These results are concordant with previous studies in other late-onset diseases. We present some possible explanations and propose future studies, namely in terms of family system and individual life story that might enable us to understand these apparently optimistic findings.

EP36. Costs and Benefits of a Pre-Symptomatic Testing

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Machado-Joseph disease is a neurogenetic disorder, beginning late in life, with a dominant pattern of transmission .
We have constructed a self-response inventory to evaluate the perception of costs and benefits of the Machado-Joseph disease pre-symptomatic test.This inventory was administered to 44 individuals at-risk for Machado-Joseph disease.
First, we evaluated the discriminant capacity of the *itens*: its reliability was satisfactory, with a Kuder-Richardson coefficient of .614 for the 16 *itens* version and .76 for the 13 *itens* version, and a even-odd

correlation of .649 for the total version of the inventory. The small version of the inventory had an even-odd correlation of .493. Factor analysis showed a bidimensional structure: “perceived costs of pre-symptomatic testing” (5 *itens*) and “perceived benefits of pre-symptomatic testing” (8 *itens*). The total score shown by both factors was 43.8%.
Social desirability was controlled with success by the correlation between the total scores of our inventory and those of the Marlowe and Crowne “Social Desirability Scale”: r=-.149; p=.333.
These results suggest adequate construct validity, once these tests assess different attitude components.
The convergent validity was confirmed by correlation studies with three scales - “Scale of Attitudes Facing Doctors and Medicine”, “Scale of Perception of Emotional Overload Associated with Genetic Disease” and “Scale of Acceptance of Pre-Symptomatic Testing”.
This inventory thus seems to be an adequate instrument to assess important subjective dimensions (expectations) involved in the decision-making process during pre-symptomatic testing for Machado-Joseph disease.

EP37. Psychological Well-Being in individuals requesting presymptomatic testing for late-onset neurological diseases

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Psychological issues are important for counselling and intervention in hereditary late-onset diseases (Huntington disease, Machado Joseph disease and familial amyloid neuropathy), namely in the process of adjustment to the results of genetic testing.
Our aim was to evaluate psychological wellbeing in persons coming for pre-symptomatic testing, when compared with the general population. The “Psychological General Well-Being Schedule” (PGWB) was developed for the purpose of providing an index that could be used to measure self-representations of intrapersonal affective or emotional states reflecting a sense of subjective well-being or distress.
The control group was chosed from the general population: mostly students and some health and business professionals.
There were significative differences when we compared the group of individuals at-risk and the control group: the group at-risk presented less anxiety and depressed mood and more positive wellbeing, vitality and auto-control, and showed higher psychological well-being indicators.
One may have expected that individuals at-risk that came for pre-symptomatic testing were more concerned about their health and showed more adverse indicators regarding their psychological well-being, since they were more aware of the risk of being a carrier for one of those diseases. Our results, however, proved otherwise: the subjects at-risk showed better indicators of psychological well-being than the controls.
Face to these results, we may suggest two possible explanations: (a) a defensive and denial attitude from the group of individuals at-risk, and/or that (b) these are psychologically more resilient, what may have motivated adhesion to pre-symptomatic testing, through their own auto-selection.

EP38. Towards valuing outcomes and preferences for clinical genetics services

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In clinical genetics, outcome measurement is problematic. “Patients” in clinical genetics are generally healthy: “We dispense words not tablets” (Clarke 1997, p.165). A variety of approaches have been attempted, none of which have proved adequate e.g. recall of risk figures, reproductive plans/behaviour and patient satisfaction.
It is generally recognized that because of the nature of genetic disease, that traditional approaches to outcome measurement will not be relevant, and there have been calls for development of new measures. Systematic literature reviews of outcome measures and models of service delivery in clinical genetics are underway, and are informing this research. Recent evidence suggests that aspects of the client/counsellor encounter may contribute significantly to a good outcome for clients, such as empathy, trust and rapport, two-way communication, and responsiveness to consumer needs. To validate and extend the list of attributes identified to date, a series of 8-10 focus groups with both users and service providers are planned for March 2004, for both cancer and general genetics services. The focus groups will be run by a trained facilitator, tape-recorded and transcribed, and analysed using the constant comparative method. This paper will present the findings. Attributes identified by the analysis will be ranked, and used to design measurement tools to value users’ preferences for general genetics services and services for familial cancer.
Clarke AJ. (1997). Outcomes and process in genetic counselling. In: P S Harper and A J Clarke (Eds), *Genetics, society and clinical practice* (pp165-178). Oxford: Bios Scientific

EP39. An overview of results of non-verbal techniques in psycho-educational groups in genetics.

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Psycho-educational group work is a useful source of support in addition to face-to-face counselling in the different fields of medical genetics, like prenatal diagnosis, oncogenetics, neurogenetics, and cardiogenetics. It enables participants to share their emotions with people in the same situation and to clarify their own decision-making process. Moreover, it facilitates the process of coming to terms with the diagnosis and course of the disease. The presence of a professional group worker contributes to a well-balanced programme: from ensuring a free exchange of experiences and opinions, to providing a more structured approach for sensitive topics.
Social workers in clinical genetics in the Netherlands organise psycho-educational groups for hereditary breast and ovarian cancer (women at risk and patients), for Huntington’s disease (separate groups for gene carriers, patients, and partners of patients) and for couples after termination of pregnancy. In addition to the verbal exchange of experiences, information and emotions, non-verbal techniques are used to encourage participants to deal with emotional subjects. For example, the family coat-of-arms is one technique for making people aware of the underlying (often unspoken) norms and values which govern the family in different generations; another example is building self-portraits as a group exercise. Using this specially developed material, it is possible to reveal the behavioural, cognitive and emotional factors and make them tangible in concrete situations.
The poster shows an overview of different non-verbal techniques. We present a description for using the techniques for the group worker and the participant and illustrations of results made by participants.

EP40. Between choice and moral responsibility: decision-making in genetic counselling

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Healthcare encounters generally invoke moral and psychosocial dimensions to the extent that a critical understanding of the patient’s social life needs to be encompassed within the agenda of both service delivery and research. When an illness is recognised as genetic, it inevitably assumes a familial status. It thus becomes imperative for genetic counsellors to create a framework for clients’ understanding of their moral duties and responsibilities, while preparing them for coping with `risks of knowing’ vis-à-vis `risks of occurrence’. Beginning with a distinction between principle-based and consequence-based approaches to ethics/morality, it is suggested that both these approaches merge in the contingencies of the counselling encounter. The offer of alternative choices - which derives from an ethos of non-directiveness - invariably borders on moral and psychosocial issues, leading to shifts in the framing of available action-scripts. Drawing on clinic data transcripts from an ongoing Wellcome Trust funded project, we examine `socio-moral’ accounting practices which underpin the decisions about genetic

testing and disclosure of test results along the lines of: (i) Reasoning (rationality); (ii) Responsibility (including blame allocation, other- and self-orientation); and (iii) Role-relationships.

EP41. Cognitive structures of risk perception : could concepts of cognitive behavior therapy facilitate the genetic counselling process? A theoretical exploration as a basis for future research in intervention studies

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So far research has concentrated to evaluate states of anxiety and depression regarding clients who undergo presymptomatic testing for an incurable autsomal dominant neurodegenerative disease. We have learnt that presymptomatic testing in the symptom free period, does rarely lead to exceptionally high scores on anxiety and depression scales when compared to the general population. (Wiggins, S. a.o 1992, Tibben, A. a.o. 1993, Hayden M.R.a.o. 1995, Almqvist, E.W. a.o.1999, Codori,A.M.a.o. 1997, Tibben, A.a.o. 1997, Wahlin, T.B. a.o.1997).
Since anxiety and depression are feeling states and since the test population does not show a very significant difference from the general population, we have collected data on cognitions from clinical experience with clients at risk for various incurable diseases (Huntington's Disease, Cadasil en HCHWAD) . We have identified three different cognitive structures that clients use to interpret their risk status:
- the cognition that emphasizes the chance aspect
- the cognition that emphasizes a particular outcome
- the cognition that emphasizes the existential aspect
These structures will be described in detail. We assume, such as the theorists of Cognitive Behaviour Therapy, that the way one thinks about the risk will influence how one feels about this dilemma. We are using the information processing research on the relation between emotion and cognition and Beck's developed schema model of cognitive processing for the further understanding of cognition - emotion interaction (Beck 1976, Beck A.T. & Emery, G. 1985.) With this theoretical exploration as a basis, suggestions will be made how further research could explore the relevance of cognitive interventions and to what extent these interventions could facilitate the genetic counselling process.

EP42. General public's knowledge, interest and information needs related to genetic cancer, an exploratory study

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Group interviews were conducted with 49 people to get an idea of what and how the general public thinks about genetic cancer. Understanding what people think and need is crucial for adequate public health communication about genetic issues.Group discussions revealed that participants believed that the vulnerability for cancer was largely depended on their life style, also if cancer ran in the family. People found it difficult to distinguish cancer from genetic cancer since in both cases the cause was related to cell problems. In general participants expressed lacking adequate knowledge of genetic cancer, which was also confirmed by the revealed misconceptions during the discussions. People mentioned both advantages (knowing one's risk, perform preventive actions, more openness, less taboo, and more knowledge) and disadvantages (fear arousal, difficult to time, undirected, tenability) of receiving genetic information. Although people felt ambivalent about wanting to receive information about genetic cancer, as yet the general tendency seemed to postpone opening up to genetic information until there was a relevant case in the family. Subsequently, the preferred information sources were family members and health professionals. According to the participants mass media should provide information on relevant features of genetic cancer to look out for. As yet, people showed little interest in more biological genetic information.

EP43. Genetic counselling and diagnostics: Opinions of counsellees, professionals, and members of lay organisations

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Diverse institutions are interested in using results of genetic diagnosis. What are the opinions of various groups regarding problems in connection with genetic diagnosis? A questionnaire (10 questions, 3 possible answers) was sent to at least 13 groups (N=1,017).
Results: 1) Except for medical students in their 4th semester more than half in all groups agree that a genetic counsellor should speak about other genetic risks if counsellees wish it. 2) There are different opinions between the groups with regard to active counselling. 3) All groups agree that genetic diagnostics only should be carried out within the frame of counselling. 4) They also agree that a healthy person should decide for himself whether to undergo genetic analysis. 5) More than half in all groups agree that gene analysis of healthy children should only be carried out for prevention. 6) Only physicians, cancer families and large numbers (80%) of members of the Huntington's association agree that employers/insurance companies should never be informed about results from genetic analysis. 7) Except for medical students in their 4th,5th semesters more than half of members of the other groups agree that prenatal diagnosis should only be offered in cases of higher risk. 8) Most members of the Huntington and Heredoataxia association, accompanying persons and counsellees would accept termination of pregnancy, if there was an embryopathic indication. 9) Only a few persons in all groups think that PGD should be forbidden . 10) Only students of midwifery think that genetic manipulation should be forbidden(> 70%).

EP44. An evaluation of the Psychosocial Impact of MaMmographic Surveillance services in women under 50 at moderate or high risk of inherited breast cancer (PIMMS)

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In many UK centres, mammography is being routinely offered to women under 50 at moderate/high risk of breast cancer. The clinical efficacy of screening in this population has not been proven and is currently being evaluated in an on-going study (FH01). PIMMS is a complementary study which is exploring the psychological impact of screening in this group of women.
The main study aims are:
• To describe the psychological impact of undergoing mammography
• To assess the roles of appraisal, coping, decisional conflict and personality type in predicting the psychological impact.
• To provide a qualitative understanding of the experiences of women who take part in screening
• To record uptake rates and the proportion of women who have made an informed decision to have a mammogram
PIMMS is a multi-centre cohort study using both qualitative and quantitative techniques:
• A questionnaire will be sent to 3,000 women prior to mammography
• A follow-up questionnaire will be sent to women 2 weeks after their final result and again six months later
• Face-to-face interviews will be conducted with a sample of women receiving an initial clear result and a clear result following further assessment (false positive). Women with screen detected and interval cancers will also be interviewed.
Data collection began in May 2003 and will continue for a three year period.
Results will be used to optimise the service provision of mammography for women under 50 at moderate/high risk of breast cancer because of a family history.

EP45. Knowledge about genetics in diverse populations and associations with attitudes towards genetic testing

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Background: Several studies revealed associations between the extent of genetic knowledge and general attitudes towards genetic testing as well as willingness to undergo genetic testing. Our study aimed to explore the genetic knowledge in diverse populations.
Method: Furr and Kelly (1999) developed the Genetic Knowledge Index (GKI), which is a five items instrument for the assessment of general knowledge about genetics. In our work, the German version of the GKI had shown inadequate psychometric properties. Therefore, we developed the „Genetischer Wissensindex“ (GeWI), a 12 items self-report instrument with a dichotomous true/false response mode, and tested its usefulness in different samples with more than N = 1.000 participants. We included medical students, other students, physicians, members of self-help groups, patients and persons from the general population.
Results: The GeWi showed good psychometric characteristics (reliability r_{tt} = .77, for validity issues see Berth et al. 2004). Medical students indicated highest knowledge, while members of the German Huntington self-help group had least knowledge. Neither age nor gender or religious affiliation showed associations with genetic knowledge. We analyzed associations between knowledge and attitudes towards genetic testing in a subsample of students. No significant associations were found.
Conclusion: Our study underlines that importance of considering consultants‘ level of knowledge in genetic counseling. Counselors should explore clients‘ genetic knowledge to provide comprehensible information. The GeWI might be a facilitative instrument in this process.

EP46. Quality circles in prenatal diagnostics counseling as an example for interprofessional cooperation

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A pilot study by the Bundeszentrale für Gesundheitliche Aufklärung (German Federal Centre for Health Education) has revealed a lack of cooperation between medical doctors and social workers during counseling in the context of prenatal diagnostics. This led to the hypothesis that both professional groups tend to distrust one another’s counseling skills. A new project started in 2003 introducing interprofessional quality cirles in (the areas of) Freiburg, Heidelberg and Mannheim.
These circles on prenatal diagnostics are intended to establish a comprehensive counseling network for pregnant women and their partners. The latter are legally entitled to professional counseling according to German law.
Here we report on the Freiburg circle. It serves as a representative example to describe first positive experiences, to depict the workings of the network structure and to present guidelines that have been established to improve the quality of further co-operation.

EP47. Decision making towards prophylactic surgery in women at high risk for breast and ovarian cancer susceptibility

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A family history of breast and ovarian cancer is associated with an increased life time risk for breast cancer up to 80% and for ovarian cancer up to 40%. Women at high risk of breast cancer is recommended an intensified surveilllance program for early detection. However for those women who want to reduce cancer risk, chemoprevention which is rarely accepted and propyhlactic surgery are possible preventive options. Uptake and decision making of prophylactic surgery in women at high risk for breast-ovarian cancer susceptibility has not been examined for German high risk persons so far.
In a folllow up survey (standardized telephone interview) high risk women were asked about their attitude and uptake of preventive surgery with a median follow up of 2,5 years after having received interdisciplinary counseling and genetic testing. Predictor variables were personal risk, doctor´s advice, severity, risk perception, cancer worry, cognitive and emotional aspects of intensified surveillance. Uptake of prophylactic surgery was very low. 7% of high risk women decided for prophylactic mastectomy, 8% underwent prophylactic oophorectomy (N=293). Predictors for prophylactic mastectomy were having breast cancer, number of relatives with breast cancer in the family, internal health locus of control and doctor´s advice. Prophylactic oophorectomy was predicted by age over 40, family history of ovarian cancer and doctor´s advice. Considering prophylactic mastectomy was predicted by higher education, importance of doctor´s advice, risk perception and cancer worry. Intention to decide for prophylactic oophorectomy was associated with a family history of ovarian cancer, doctor´s advice and cancer worry.

EP48. Genetic counselling summary letters: patients’ views and uses

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Writing summary letters to patients after genetic counselling consultations is standard practice in most Centres, yet little evidence exists on patients’ reactions to, and use of, these letters. This study investigated patients’ views and long term use of genetic counselling summary letters for a wide range of genetic referrals. 205 individuals were sent a questionnaire 1 (n=103) or 5 (n=102) years following clinic attendance. These included patients attending for a wide range of consultations including cancer, dysmorphology and neuromuscular assessment. 57/205 questionnaires were returned completed (response rate 28%). 96% of respondents found the letter “easy to read”. When asked to describe the most helpful aspects of the summary letter, 52/57 responded with a range of themes including: explained/clarified/summarised their situation, written information about probability easier to understand and to refer back to, the personal style of the letter, aid to explain situation to others, emotional validation and increased confidence, and a tool for future reference.14/57 respondents described unhelpful aspects of the summary letter, including missing information and length of time before letter received. 97% of respondents (1 and 5 year groups) still had their summary letter in a “safe place”. Nearly 80% of respondents in both the 1 year and 5 year group had re-read their letter, often several times. 83% shared their letter with at least one other person including partners (most commonly) relatives, friends and professionals. Letters were shared to help others understand the patient’s situation, rather than just to transmit relevant genetic information to relatives at risk.

EP49. The dilemmas of carrier testing for a rare recessive disorder: a case study

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Individuals with a family history of a rare recessive disorder are often referred for genetic counselling and testing for carrier status. Advances in genetic knowledge has meant that carrier testing is a theoretical option for an increasing number of disorders, and today’s clients have a growing expectation that testing will be possible and available.
Carrier testing for a rare recessive disorders can be expensive, and then testing partners with no family history may be inconclusive. This case study explores the dilemmas encountered when a family of young adults are referred for carrier testing for galactosaemia. A 52 year old aunt, still alive, was diagnosed with the condition at birth. Her sister, mother of the referred adults, was found to be a carrier 25 years ago. The referral raises complex issues: the conflict between the expectations of the family and the constraints of the service; the ethical dilemmas involved in obtaining consent to confirm the

diagnosis in an adult with learning difficulties and the counselling difficulties in explaining complicated genetic information and helping individuals to decide how best to proceed. The study demonstrates the challenges in having a policy as regards carrier testing for rare disorders if we are to respond to the expectations of our clients.

EP50. The psychosocial impact of genetic testing, on parents of children previously clinically diagnosed with a neurodevelopmental genetic disorder.

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Recent molecular genetics advances now make it possible to confirm the diagnosis of many genetic disorders, which were previously based on clinical evidence. As a result, many families find themselves faced with having a genetic test, following a number of years living with a clinical diagnosis. It has been assumed that these genetic tests are beneficial to families, however, there is no known research into the impact that this has on families. The aim of this study was to investigate the psychosocial impact of genetic testing, on parents of children previously clinically diagnosed with a neurodevelopmental genetic disorder.
Using qualitative research methods, 6 parents of children with Rett or Angelman syndrome were interviewed and their experiences of the clinical diagnosis and genetic test were explored. All parents valued the clinical diagnosis as it helped to explain their child's problems to others and also benefited their child's care. While the genetic test was also found to have a positive impact on parents, for example, relieving guilt and uncertainty, for some parents it also caused confusion about the implications of the test results.
The psychosocial impact of both the clinical diagnosis and the genetic test will be explored and discussed.

EP51. Women's attitudes to prenatal testing and termination of pregnancy across a range of conditions: patterns and explanations

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Advances in DNA technology mean that prenatal tests for a wide range of conditions may soon become available. Obtaining separate informed consent for each is likely to be impractical. This study explored the extent to which individuals' attitudes towards testing for different conditions cluster together and might be used as a basis for an alternative, grouped, consent procedure.
Participants were 420 postnatal women in the north of England. Each completed a questionnaire that presented them with a hypothetical scenario of a diagnostic test early in pregnancy and brief descriptions of 30 conditions. They were asked to say for each whether they would want testing and whether they would consider termination of pregnancy. Only 25% of women said that they would want testing for all the conditions, and 3% said that they would want none. The majority of the sample were therefore indicating that they would want some tests but not all.
Responses were compared between groups and a classification system of conditions was developed using factor analysis and other statistical techniques. The acceptability of the classification system was assessed in 8 focus groups with maternity service users, service providers and voluntary organisations.
Sixty women were also interviewed about the reasoning behind their attitudes. This paper will draw on both the quantitative and the qualitative data to discuss the groupings and variations in responses to the different disorders, as well as focus group participants' views of the acceptability of the classifications for consenting to prenatal testing.

EP52. Multidisciplinary care for inherited cerebellar ataxias

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Inherited ataxias are characterised by progressive loss of co-ordination and speech. Sometimes the genetic basis is known but treatment is not yet available. We proposed a multidisciplinary care, including neurology, physical medicine, physiotherapy, speech therapy, psychological interviews with the patient and caretakers, and genetic counselling. All interviews and examinations were performed on the same day and place followed by conclusions given after interdisciplinary consensus to the patients and caretakers.
From February 2003 to December 2003, 55 patients participated, including 18 with autosomal dominant cerebellar ataxias, 14 with Friedreich ataxia, 2 with multiple system atrophy of the cerebellar type, 4 with spastic paraparesis and 17 with genetically undetermined familial cerebellar ataxias. Mean age at examination was 47.5 years (range 17-80) and mean disease duration 14.7 years (range 2-39). Active physiotherapy was initiated for the first time in 12 patients and reinforced in 17. Technical aid was proposed in 18 cases, including wheelchair for the first time in 3. The most striking finding was the high frequency of mood disturbances with current depression (n=28) and anxiety (n=4). Antidepressant was initiated in 24 patients and psychotherapy in 29. Satisfaction was evaluated by questionnaires and telephone calls during follow -up.
We conclude that in progressive neurological diseases without curative treatment the usual care with a single annual neurological visit may be insufficient and multidisciplinary care helps to detect specific needs. The consensus of all participants with different skills and the time given to the discussion with the patient are helpful in these devastating conditions.

EP53. Huntington's Disease: Description of the clinical image in a nursing home setting

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Background: Huntington's disease (HD) is a hereditary progressive neuropsychiatric disorder, characterized by involuntary movements, personality changes, and cognitive deterioration. Little is known about the relationship between clinical manifestation, premorbid personality and intimate/social relationships. To improve individually tailored programs of the nursing of patients with HD we study the relationship between clinical manifestation in the later stages, the patient's premorbid personality, and the quality of social relationships before becoming ill and in the first stages of the disease.
Sample: Five nursing homes with specialized wards for HD (4 Dutch and 1 Belgian) care for 92 inpatients and 19 outpatients. A contact person of each patient, familiar with the premorbid personality and intimate and social relationships of the patient, is interviewed after consent. The participants administer questionnaires.***Measures***: The Behaviour Observation Scale Huntington (BOSH) has been developed for the description of the nature and course of HD in the later stages. The BOSH, the Unified Huntington Disease Rating Scale (UHDRS), and relevant medical data are administered from the medical files. A semi-structured interview (about 1½ hours) with the contact person contains questions on the patient's (early) experiences with HD, intimate and social relationships, work and hobbies. The questionnaire for contact persons contains an adaptation of the NEO-FF-I (personality characteristics), the Social Support List, the Hospital Anxiety and Depression Scale, and a selection of items of the Impact of Event Scale.
Results: Preliminary results of the functioning and characteristics of the patients, and the relationship with premorbid personality and intimate/social relationships will be presented.

EP54. Emotional experiences and representations associated with the psychological development of pre-symptomatic individuals with familial amiloyd polyneuropathy, type I (FAP-I) - Portuguese, Andrade

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Individuals who live in a psychological environment threatened by disease/loss/death of a parent organize their reactions to psychic suffering and emotional expression according to their life experiences during childhood. **Objective**: To understand the experiences and emotional representations associated with some aspects of psychological development of individuals who come for pre-symptomatic testing. **Method:** We conducted clinical interviews, using an anamnesis guide, with 78 individuals at risk (31 men and 47 women), aged 17 through 70 (mean 25.8 years), and with various stages of education. The following items were analysed: own evaluation of childhood and memories, diseases, dreams and graphic expression, psychomotor development, breast-feeding, separations/ changes, relationships. **Results**: The majority of individuals claimed to have had a "normal/good" childhood; about 1/3 of individuals describe symptoms of somatic expression in childhood; the majority of individuals did not remember their dreams, or remembered dreams with a threatening content; almost all acknowledged having been breast-fed; almost all initiated a stable relationship in the final period of adolescence, and usually got married quite soon. **Conclusion**: FAP possibly leads these families to develop family-cohesion mechanisms that give them the perception of proximity/support/ safety, which generates the idea of a "good/normal" childhood. These individuals do not explicitly express psychological suffering. Repression of oniric expression and tendency to somatize suggest a blockade of symbolic activity. The probability of these individuals suffering from FAP may lead them to anticipate relationships, marriage and other life aspects.

EP55. Impact of coping style on psychological distress in women at increased risk of developing breast cancer

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Background The MRISC study evaluates the psychological implications of a regular breast cancer surveillance program for women at different levels of increased risk of developing breast cancer. Attending a surveillance program may cause elevated levels of psychological distress. Coping style can act upon the degree of reported distress.

Objective Impact of coping style on the level and course of general and breast cancer specific distress will be examined, as well as differences of this impact between the three risk categories (15-30%, 30-50%, 60-85%).

Method Before and after two consecutive surveillance appointments, participants completed several psychometrically tested questionnaires. The Hospital Anxiety and Depression Scale (HADS) assessed general distress and the Impact of Event Scale (IES) assessed breast cancer specific distress. Different coping styles were assessed by the Utrecht Coping List.

Statistical analysis The impact of coping style on distress was analysed by structural equation modeling. The course of distress, was analysed by piecewise regression modeling.

Results 351 Women with a mean age of 40½ years (range 21 - 63) participated. Of the different coping styles, only the depressive style substantially affected the levels of general and breast cancer specific distress (all p-values < 0.01). However, the courses of distress were unaffected by the depressive coping style. The findings will be visualised.

Conclusion The results suggest that particularly the depressive style of dealing with problems elicits a high level of psychological distress. This implies that it is of clinical importance to offer professional support tailored to this way of dealing with problems.

EP56. World Wide Web: confusing people or a possible way to help? A genetic experience in giving information

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In the last years genetics has enormously expanded its boundaries and many persons look for information on the Net.
Created in 2001genetica.soloinrete.it is an Italian web site with the aim to satisfy the growing need of information. A team of experts with PhD in Genetics assists navigators not confident with English language. The site acquired HON certification in 2003.
The site is divided in sessions each dedicated to a specific topic:
-Genetics and School is mostly used by students or people in search of the latest news or any other kind of general information on genetics;
-Genetics and Pregnancy gives help to future parents facing problems of genetic diseases in prenatal diagnosis and in the interpretation of results of genetic tests.
-Genetic and Diseases assists people dealing with specific genetic diseases.
The Site further can also supply a list of centres of diagnosis or treatment which can be consulted. Experts doesn't make any diagnosis by e-mail.
During these two years of activity the site has received an average of 150 visits per day and a total of 900 questions. 45% of questions were for Genetic and Pregnancy, 30% for Genetic and Diseases and 25% for Genetic and School.
A database of answers is freely available from navigators. The site make consultable only those answers for which received the authorization for publishing from the questioners.
The present work analyzes the site activity to understand people needs and the psychosocial impact of the information given.