ESHG Abstracts

Plenary Sessions

PS 1. Plenary Session 1

PS01. The Human Genome Sequence: will it come to an end ?

J. Weissenbach;
Genoscope and CNRS UMR-8030, Evry, France.

The International Human Genome Sequencing Consortium has not curtailed its efforts with the annoucement of the completion of the draft sequence (June 2000). On the contrary significant progress has been accomplished since the analysis (February 2001) and the hope for a complete sequence for 2003 is still realistic.
The draft sequence has been improved by doubling the number of shotgun reads of the BAC clones included in the assembly (about 30,000 clones). This resulted in larger assembled contigs which could be more frequently ordered and oriented. Finishing is the major focus at present.
To date chromosomes 14, 20, 21, 22 and Y are fully sequenced. The published draft sequence (assembled September 2000) contained some 220,000 sequence gaps among which about 2000 were not bridged by cloned DNA fragments. The sequence gap number has decreased to 98,000 (assembled August 2001) and the number of clone gaps has been reduced to 390 (November 2001). Similarly the global coverage has increased from 2,700 Mb (September 2000) to 2,900 Mb (August 2001). 2,048 Mb of non-redundant sequence (64%) is in the finished state.
Because of the high redundance of the number of BAC clones that were used for the draft sequence, a large number of sequence variants could be identified and more than 4,000,000 SNPs are presently featured in dbSNP. Sequence analysis does not reveal similar spectacular changes. The gene count remains controversial. However the number of 25,000 confirmed genes is not going to increase rapidly since it is dependent on experimental validations.

PS02. Gene therapy of inherited disorders. Results and perspectives

A. Fischer;
Unité d'Immunologie et d'Hématologie Pédiatriques and INSERM U 429 - University Hospital Necker-Enfants Malades, Paris, France.

Gene therapy is an attractive option for a number of genetic disorders. Genetic supplementation could in theory lead to long lasting disease phenotype correction. However, efficient targeting, induction of long lasting transgene expression and a few other issues limit present application. Given the status of gene transfer technology, two settings appear more favourable. The first relies on a growth advantage conferred to transduced cells by transgene expression combined with cell longevity. This is best examplified by the severe combined immunodeficiency (SCID) condition. X-linked (SCID) is caused by mutations of the gene encoding the γc receptor subunit. Induction of expression on lymphocyte progenitors by retroviral mediated gene transfer leads to tremendous cell proliferation resulting in the generation of a high number of mature, long lived T lymphocytes. Based on this strategy, seven out of 8 patients with typical XL-SCID have benefited from gene therapy. They have indeed recovered a functional immune system with a follow-up up to 3 years without adverse effects. A dozen of other genetic conditions might therefore also benefited form this approach. The second favourable setting is based on continuous secretion of a protein in blood stream by transduced cells of various sources such as hepatocytes, fibroblaste or muscular cells. AAV vectors appear well-suited. As based on preliminary results, application to the treatment of inherited hemostasis disorders (hemophilia) or lysosomal storage disorders can be considered. Broader application of gene therapy is awaiting progress in gene transfer technology. The advent of lentiviral vectors, enabling transduction of non cycling cells, if proven safe, might provide a significant boost to gene therapy.

PS03. Patterns of human meoitic recombination

A. J. Jeffreys, K. Holloway, L. Kauppi, C. May, R. Neumann, T. Slingsby, T. Taylor;
University of Leicester, Leicester, United Kingdom.

To analyse the fine-scale distribution of meiotic recombination events in human chromosomes, we have developed PCR methods to detect crossovers in sperm DNA. Linkage disequilibrium (LD) and sperm analysis within the MHC class II region show that crossovers are heavily clustered into 1-2 kb wide hotspots that profoundly influence LD patterns, with blocks of strongly-associated markers 10's of kb long lying between clusters of hotspots. Current evidence suggests that this mosaic pattern of recombining and non-recombining DNA is quite common elsewhere in the human genome. Other similar-width hotspots identified by sperm analysis have been found closely associated with minisatellites, where they appear to drive repeat DNA instability, and in the recombinationally active pseudoautosomal pairing region PAR1. While most hotspots engage in fully reciprocal crossover, one MHC hotspot shows reciprocal crossovers mapping to different locations. This asymmetry is most simply explained by SNPs influencing the efficiency of crossover initiation, with the result that markers near the site of initiation undergo biased gene conversion as a result of gap repair during recombination. This model is further supported by the occurrence of frequent conversion events without crossover at the centre of at least one hotspot. The emerging picture is that crossovers initiate at extremely localised sites in human chromosomes, and that the similar widths of hotspots may simply reflect similar processes of gap expansion and repair operating at different hotspots. Paradoxically, these hotspots appear to be prone to extinction by meiotic drive of variants that suppress recombination activity.

PS 2. Human Genetics in the European Context

PS04. The Role of the European Parliament in Human Genetics

R. Goebbels;
Member of European Parliament, Brussels, Belgium.

Mr. Goebbels is Member of European Parliament. Former President of the Temporary Committee on Human Genetics and other new technologies of modern medecine set by the European Parliament in 2000-2001.

PS05. The European Orphan Drug Legislation: Impact and Issues

Y. Le Cam;
EURORDIS, Plateforme Maladies Rares, Paris, France.

Mr. Y. Le Cam is General Director of EURORDIS. The European Organization for Rare Disorders (EURORDIS) is a coalition of more than 200 associations and National Alliances of patients organisations associated with rare diseases from 14 countries in Europe.

PS06. Patenting and Licensing diagnostic tests: the BRCA case

Gert Matthijs[1], D. Stoppa-Lyonnet[2];
[1]Leuven, Belgium, [2]Paris, France

PS 3. Genetic Testing in Minors

PS07. Neonatal screening for CF: a 13 years experience in Brittany (France)

C. Ferec, V. Scotet, M. de Braekeleer, M. P. Audrézet;
EMI-U 0115 - C.H.U., Brest, France.

Cystic Fibrosis is the most frequent autosomal recessive disease among Caucasians. The disease, characterized by chronic broncho-pneumopathy and pancreatic insufficiency, is still fatal with a median life expectancy of about 30 years. Among the possible strategies for prevention is neonatal screening based on the expectation that early diagnosis leading to early treatment would result in lower morbidity and longer life expectancy. Thirteen years ago, we started a systematic neonatal screening program in Brittany, France, a region of 2.8 million inhabitants mostly of Celtic origin. The initial program was based on an IRT/IRT two-tier protocol (1989/1992) then we implemented a two-tier IRT/DNA analysis pilot program. During this 13 years period, 454 285 IRT tests were done on children born in Brittany and 163 were diagnosed with CF. The cumulative incidence of CF was one in 2787. The number of new cases identified each year is relatively constant (12/14). All the mutated CFTR alleles but one were characterized: 41 different mutations were identified corresponding to 48 different genotypes. During this period, 35% of these one-in-four risk couples opted for prenatal diagnosis and

during the same period 10% of CF children were diagnosed *in utero* due to the discovery of an hyperechogenic bowel during pregnancy. We evidenced in this population study the spectacular changing epidemiology of CF
The possibility of direct benefits resulting from neonatal screening (with nutritional and respiratory benefits) raises the question of whether the time has arrived for routine neonatal screening for CF. We have shown the efficiency and the feasibility of neonatal screening for CF in Brittany based on 13 years experience. The IRT combined with mutation analysis on the same Guthrie cards can be done in any population in which most of the mutations can be identified. The implementation of a national neonatal screening program for CF has been decided by the health care authorities in France, the program has begun at the beginning of this year.

PS08. Lessons from the newborn screening programme in Wales

A. J. Clarke, E. Parsons, D. Bradley;
Department of Medical Genetics, University of Wales College of Medicine, Cardiff, Wales, United Kingdom.
The newborn screening programme in Wales has incorporated tests for Duchenne muscular dystrophy (DMD) since 1990 and for cystic fibrosis (CF) since 1997. Initial concerns that screening for DMD might lead to family disruption have not been justified, but we have learned lessons about screening for diseases where the affected child is not expected to benefit from early diagnosis.

1. The test must be perceived as optional by the midwife and family.
2. This requires an educational initiative for the community midwives.
3. Changes to service delivery may make more explicit the optional character of such non-therapeutic screening tests.
4. A protocol for handling positive screening test results must be developed and monitored.
5. Careful and sustained coordination of communication between the family, the primary health care team and specialist care is required.

Newborn screening for CF also identifies unaffected carrier infants, who will not benefit from their early diagnosis – they have elevated trypsin levels, one recognised *CFTR* mutation but normal sweat electrolytes. The recognition of these infants may be regarded as a disadvantage of screening, but does result in staging of the 'bad news' concerning affected infants. Should only those infants with two *CFTR* gene mutations be regarded as positive on the screening test ? Our experiences have implications for the introduction of screening for additional disorders, such as MCAD deficiency. We have also found that a few infants have in the past been missed by the routine newborn screening programme. Newborn screening is not just a laboratory process.

PS09. Genetic Testing for Hereditary Colorectal Cancer in Children: Long-Term Psychological Effects

A. M. Codori, K. L. Zawacki, G. M. Petersen, D. L. Miglioretti, J. A. Bacon, J. D. Trimbath, S. V. Booker, K. Picarello, F. M. Giardiello;
Johns Hopkins Hospital, Baltimore, MD.
Children who carry a gene mutation for familial adenomatous polyposis (FAP) need annual screening for precancerous polyps and eventual cancer-preventing colectomy. Predictive genetic testing can identify children who need regular screening. Testing children for FAP has clear medical benefits, but the psychological effects have not been well studied. We evaluated the long-term psychological effects of genetic testing in 48 children and their parents. In each family, one parent was a known gene mutation carrier. Before genetic testing, and three times afterward, participants completed measures of psychological functioning, which, for children, included depression and anxiety symptoms, and behavior problems and competencies. Parents completed a measure of depression symptoms. Data were collected at 3-, 12-, and 23-55 months after disclosure. 22 children tested positive; 26 children tested negative. Mean length of follow-up was 38 months. There were no clinically-significant changes in mean psychological test scores in children or parents, regardless of the children's test results. However, children who tested positive and had a mutation-positive sibling showed significant, but subclinical, increases in depression symptoms. Furthermore, several individual mutation-negative children with a positive sibling had clinical elevations in anxiety symptoms. Behavior problems declined for all groups, and behavior competence scores remained unchanged. We conclude that most children do not suffer clinically-significant psychological distress after testing. However, because some children showed clinically-significant anxiety symptoms, long-term psychological support should be available to those families with both mutation-positive and -negative children, and with multiple mutation-positive children. Our findings call for a multidisciplinary approach to genetic testing for children.

PS10. Carrier Testing in Childhood: Conflict or Compromise?

C. Barnes;
Genetics Centre, Guy's & St. Thomas' Hospital Trust, London, United Kingdom.
The advantages of performing a diagnostic genetic test, with proven medical benefits, on a symptomatic child is seldom questioned. Equally, there is a general consensus against testing healthy children for untreatable adult-onset genetic disorders. Genetic testing to determine the "carrier" status of a healthy child with a family history of a recessive or X-linked monogenic disorder or a balanced chromosome rearrangement, however, remains controversial. There are many views on this issue, and the parents' perspective can be very different from that of medical professionals, amongst whom opinions and practices vary. Also, the impact of carrier testing on children themselves remains a relatively unexplored area.
After an overview of the relevant literature to date, the response of one Genetics Centre to requests for the carrier testing of minors will be described and discussed.
A Position Statement on the genetic testing of children was devised in consultation with laboratory colleagues. This document was circulated to regional paediatricians, and supplied to other physicians on request. The document provides basic information about issues to consider in childhood testing and a summary of current departmental guidelines on the genetic testing of minors. The document explains why requests for carrier tests in children are no longer automatically accepted, and strongly encourages clinicians to refer parents requesting such tests for genetic counselling. An information leaflet for parents is also available.
It is hoped that such proactive initiatives can increase constructive dialogue between parents and professionals, and decrease the conflict that has often arisen in this area.

PS 4. Heart and Muscle

PS11. Cardiac development and Cardiovascular malformation

J. Goodship, D. Henderson;
Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne, United Kingdom.
Understanding normal development is important to improved understanding of cardiovascular malformation (CVM). Development of the four chambered heart from a linear tube is a complex process. I will present current thinking that takes information from older studies but incorporates newer data using molecular markers and 3D reconstruction. Of particular importance is the ballooning model of development of the cardiac chambers from the primary heart tube. I will then illustrate, using three examples, how mutations in genes expressed at different stages of development lead to CVM. *ZIC3* is a transcription factor with a role in early development. Mutations in *ZIC3* lead to a range of heart defects associated with laterality disturbance demonstrating the importance of early events that establish midline and the left right axis to normal cardiac development. *NKX2.5* is a cardiac specific homeobox gene and a homologue of Drosophila *tinman*. Mutations in this gene have been reported in patients with cardiac malformations and conduction defects. The third example is the mechanism by which elastin mutations lead to supravalvular aortic stenosis. Progress has been made in identifying genes implicated in CVM occurring in syndromes e.g. *TBX5* in Holt Oram syndrome, *TFAP2B* in CHAR syndrome, *PTPN11* in Noonan syndrome. However the vast majority of CVMs are isolated malformations. Diabetes is a known risk factor but other environmental factors have not yet been identified. Genetic factors are implicated as the recurrence risk is substantially higher than the population incidence but little progress has been made in identifying

these genes. Cardiovascular malformations occur in 7/1000 livebirths; the challenge is to identify their causes.

PS12. Hypertrophic cardiomyopathy: more than just a disease of the sarcomere

H. Watkins;
John Radcliffe Hospital, University Department of Cardiovascular Medicine, Oxford, United Kingdom.
No abstract received.

PS13. Molecular Mechanisms in Myotonic Dystrophy (DM1)

T. A. Cooper;
Baylor College of Medicine, Department of Pathology, Houston, TX, United States.
No abstract received.

PS 5. Late Breaking Research Session and Baschirotto Lecture

PS14. Baschirotto Lecture: Gene discovery in cancer: who benefits?

A. de la Chapelle[1,2];
[1]Human Cancer Genetics Program, The Ohio State University, Columbus, OH, United States, [2]Folkhälsan Institute of Genetics, Helsinki, Finland.
When new genes with cancer significance are discovered it is reasonable to ask how and when the discovery can be translated into cancer therapy and prevention. Unfortunately, the most common answer is that we do not know. A more suitable question would seem to be whether novel genes will have any impact at all on cancer therapy.
In the academic world the great incentive that drives researchers is - in addition to curiosity if not passion - career aspects. Researchers will be credited for discoveries they make no matter whether the discoveries are ever translated into e.g. new drugs or treatment modalities.
In the commercial world the great incentive is money. Successful discoveries directly translate into money for all: the company, its employees, scientists, and shareholders.
How about the public, the cancer patient? The patient has more at stake than the researcher or the company. Luckily, whether a discovery has an impact on the clinical outcome is the ultimate measure of its success. Therefore, the efforts of academic researchers and companies can be said to ultimately focus on the patient, and almost nothing but the patient. This is fortunate, but progress has nevertheless been dismally slow. It would appear that every time a new research area is opened, hopes of ultimate translational success are high but in reality progress is slow. The recent breakthroughs in drug development targeting genetically determined defects have instilled high hopes. Another area showing promise is the molecular screening for inherited susceptibility to cancer. Lives can be saved and efforts can be optimized when clinical surveillance can be focused on those at high risk while those at low risk can be spared. It should not take many years before we know whether presently ongoing large investments - both academic and commercial - will pay off.

Symposia

S 1. Cognition and Behaviour

S01. The study of behavioural phenotypes : implications for practice and management

A. Swillen, J. P. Fryns;
Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium.
A major challenge in both clinical practice and research in the field of mental retardation and of learning disorders is to identify the underlying causes : the genetic, chromosomal and environmental factors that have an important influence on a person's development and behaviour.
Advances in clinical genetics have lead to an increased recognition of specific syndromes. In recent years, cytogenetic and molecular genetic tools have resulted in the identification of the underlying genetic defects in a large number of these disorders.
For many years, interest has focused on the delineation of the somatic aspects of the phenotypes and their underlying pathogenetic mechanisms. However, in the last decade, researchers in this field paid more attention to the cognitive and behavioural features of various genetic conditions, the so-called "behavioural phenotypes" (B.P.).
A behavioural phenotype is "a behavioural pattern, including cognitive processes and social interaction style, that is consistently associated with, and specific to, a syndrome which has a chromosomal or a genetic etiology". This approach has proven to be of practical importance for the patients both regarding an earlier syndrome diagnosis, as well as in the multidisciplinary management and follow-up. In addition, the link between a specific behavioural phenotype and a genetic defect represents an unique opportunity to gain insight in the complex neurobiological processes underlying human behaviour. In this lecture, focus lies on the clinical goals of research in B.P. , and this will be illustrated using the Velo-Cardio-Facial syndrome (VCFS), the Smith-Magenis syndrome and the Prader-Willi syndrome (PWS).

S02. The tortuous path from genotype to phenotype: genes and cognition in mutant mice

H. P. Lipp;
Institute of Anatomy, University of Zurich, Zurich, Switzerland.
Genetically modified mice are increasingly used to analyze the relations between genes, brain and behavior. One approach concentrates on deleting or controlling genes of theoretical importance for memory and learning, e.g., genes encoding proteins for intracellular signalling pathways. The other focuses on deleting genes in the mouse homologous to human mutations causing mental retardation and cognitive impairment. Both approaches face (i) problems of elucidating the causal relations between genes, brain and behavior, and (ii) the difficulties of matching cognitive processes in mice and men.
(i) Phenotypic expression of mutation effects is often masked or altered by homeostatic regulation at the intracellular level, and by brain plasticity and environmental effects. This will be illustrated by examples taken from the analysis of genetically modified mice carrying different targeted mutations.
(ii) A cross-species comparison of cognition must take into account that the associative cortex in mice is small and largely confined to the hippocampal formation and proximally connected structures. On the other hand, hallmarks of cognition in men (e.g., general problem solving abilities and memory), and of other mammals (e.g., spatial memory and learning of rats) might be of lesser importance for mice. This will be illustrated by a comparison of cognitive impairments in the laboratory and under naturalistic conditions.
In conclusion, the availability of mutant mouse model represents a fundamental methodological progress in modeling the genotype-to-phenotype pathway characteristic for human mental retardation, but we must learn to understand the constraints imposed by species differences. Supp by SNF and NCCR "Neural Plasticity and Repair".

S03. Fragile X Syndrome – its impact on families

B. Carmichael;
Genetic Nurse Specialist, Southend Hospital, Westcliff on Sea, United Kingdom.

Fragile X Syndrome is the commonest inherited cause of learning difficulty. Although X-linked, it affects both girls and boys. While learning difficulties are a consistent feature, fragile X is also associated with characteristic behavioural problems in many affected children.
Having a child with a learning difficulty has an impact on any family, as has the presence of an inherited disorder in a family. Many of the challenges faced by Fragile X families are no different from those faced by other families with genetic disorders, or with learning-disabled children. However, there are features of fragile X which make it unique.
Some mothers of fragile X children are themselves affected, making parenting and behavioural management difficult. Women with fragile X do not always have good social skills, and this can lead to estrangement from their families and a lack of peer support. Social anxiety and lack of confidence can make it hard for some affected females seek help for themselves or for their children.
Finding the underlying genetic mechanism which causes the condition has made possible reliable diagnosis and carrier detection.

However, more than 30 years after the first observation of the fragile site on the X chromosome, prenatal diagnosis can still be problematic.
These issues will be discussed from the perspective of my work as a genetic counsellor, as an active member of the UK Fragile X Society, and from my experience of living in a Fragile X family.

S 2. Cancer Genetics

S04. Cancer gene discovery following the Human Genome Sequence

M. Stratton;
The Sanger Centre, Wellcome Trust Genome Campus, Cambridge, United Kingdom.
No abstract received.

S05. Molecular genetics of prostate cancer

T. Visakorpi;
Institute of Medical Technology, University of Tampere, Tampere, Finland.
The molecular mechanisms underlying the development and progression of prostate cancer are inadequately understood. Over the past 10 years, genetic alterations in prostate cancer have been identified using techniques, such as linkage analysis, loss of heterozygosity analysis (LOH), fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). These analyses have implicated several chromosomal regions in the development of prostate cancer. Linkage analyses and subsequent positional cloning have now revealed two prostate cancer susceptibility genes, HPC1/RNASEL, and HPC2/ELAC2. However, they seem to explain only a small fraction of the hereditary prostate carcinomas. The most common epigenetic event in the sporadic prostate tumors seems to be hypermethylation of GSTP1 gene found already in premalignant prostate lesions. The chromosomal arms that most frequently contain losses in prostate tumors are 6q, 8p, 10q, 13q, 16q and 18q. Except the PTEN at 10q23, the target genes for these deletions are not known. Although gains and amplifications of chromosomal regions are rare in early prostate cancer, they are found in late stage, especially at 7p, 7q, 8q, 18q, and Xq. Of these, the best characterized is amplification of Xq, found in 30% of hormone-refractory prostate carcinomas, and which affects androgen receptor (AR) gene. Amplification of AR gene leading to its overexpression, as well as, in lesser extent, mutations in the AR gene, seem to play critical role in emergence of hormone-refractory prostate cancer. The most commonly gained region in prostate cancer is 8q. However, the true target genes for this aberration have remained unclear.

S06. From cancer genomics to new cancer therapeutics

P. Workman;
CRC Centre for Cancer Therapeutics, The Institute for Cancer Research, Sutton, Surrey, United Kingdom.
Cancer drug discovery has now firmly entered the postgenome era. This is characterised by two features: 1) A focus on new therapeutic targets defined by the molecular pathology of cancer; and 2) Use of modern technologies, particularly genomics, high throughput screening, combinatorial chemistry, structural biology, cassette dosing pharmacology and molecular biomarkers, to increase success and accelerate the pace of drug development (see Workman P, Curr Opin Pharmacol 2 342-352 2001; Workman P, Curr Opin Invest Drugs 2 1180-1135 2001).
The expectation is that such molecularly targeted, genome-based drugs will be more effective and less toxic than cytotoxic agents and will be used chronically in long term disease management. A wide range of novel genome-based agents are in clinical and preclinical development and some have now received regulatory approval (see Workman P and Kaye SB, A Trends Guide to Cancer, Trends in Molecular Medicine, 8 (4) Suppl 2002). Herceptin (trastuzumab) is approved for treatment of ErbB2 positive breast cancer and Glivec (imatinib) for use in chronic myelogenous leukaemia and gastrointestinal stromal tumours (GIST) as a result of its action on the Bcr-Abl and mutant c-Kit tyrosine kinases that drive these diseases. Iressa (ZD1839) shows activity in various cancers through inhibition of the epidermal growth factor receptor tyrosine kinases. Hsp90 inhibitors, such as 17AAG, are of special interest because of their ability to cause depletion of multiple oncogenic proteins. Progress and challenges with the gene to drug approach in cancer will be reviewed, with examples from recent work.

S 3. Neurological Disease

S07. Friedriech and other recessive ataxias

M. Koenig, H. Puccio, P. Bomont, M. C. Moreira, D. Simon, S. Klur, M. Gribaa, C. Lagier-Tourenne, M. Schmitt;
IGBMC (CNRS-INSERM-ULP), Illkirch, Strasbourg, France.
With the exception of metabolic disorders, only two forms of recessive ataxia were well characterised some twenty five years ago : Friedreich ataxia (FRDA) and ataxia-telangiectasia (AT). DNA linkage studies allowed to identify the molecular cause of these frequent forms of inherited ataxia and to start to unravel the complex heterogeneity of the remaining cases. In a collaborative endeavour, we identified the gene defective in FRDA and its product, frataxin, a mitochondrial protein whose deficiency results in disturbances of iron homeostasis and of iron-sulfur proteins. The molecular pathogenesis of FRDA is defined by severely reduced levels, but not absence, of normal frataxin as a result of the intronic trinucleotide expansion mutation present in homozygous, and occasionally in compound heterozygous, patients. We have used conditional and inducible knock-out strategies to artificially recreate this partial frataxin deficiency in mouse models that present with several clinical, histological and biochemical features of the human disease, in different combinations. Idebenone, an antioxidant proposed by P. Rustin to protect against iron excess toxicity, showed a moderate but significant effect on survival on the first model that we tested. We pioneered the use homozygosity mapping for primary localisation of autosomal recessive conditions and applied it successfully to ataxia with isolated vitamin E deficiency (AVED), giant axonal neuropathy (GAN), ataxias with oculomotor apraxia (AOA1 and AOA2), Refsum disease (RD) and ataxia with visual impairment and deafness (van Bogaert ataxia). We subsequently identified the defective gene for the first three conditions and used the genetic knowledge to clinically delineate the AOA forms. The identification of many more genes involved in recessive ataxias should shed light on general pathological mechanisms and suggest therapeutic interventions.

S08. Friedreich's Ataxia: Insights into the Mechanism and Prospects for a Therapy

P. Rustin, V. Geromel, N. Darin, A. Munnich, A. Rötig;
INSERM U-393 - Hopital Necker Enfants Malades, Paris, France.
In 1996, mutations in the gene encoding frataxin, a mitochondrial protein of yet unknown function, were shown to cause Friedreich's ataxia, the most common hereditary ataxia with cardiomyopathy. In more than 97% of the patients, a GAA triplet expansion in the first intron of the gene results in a lack of function of frataxin, due to hampered transcription of the gene. Decreased frataxin was subsequently found to cause a generalized mitochondrial iron-sulfur protein deficiency in the heart from Friedreich's ataxia patients. Post-mortem sample analysis confirmed the functional impairment of these proteins in both heart and brain of the patients. The exact function of frataxin remains a matter of debate, the protein being possibly involved in either mitochondrial iron export or storage, or in iron-sulfur protein synthesis. Whatever the exact function of the frataxin could be, the disabled recruitment of early antioxidant defenses recently reported in patient cell lines should participate to increased sensitivity to the oxidative insults noticed in Friedreich ataxia patients. Given the strong evidences for an increased oxidative stress, antioxidant therapy appeared a reasonable therapeutic option. Patients were therefore treated with idebenone, a potent antioxidant ubiquinone analogue. A preliminary study reported a spectacular improvement of the cardiomyopathy in three patients after 6 months of idebenone oral supplementation (5 mg/kg/d). These preliminary results were afterwards confirmed on a larger cohort of patients with a significant decrease of heart hypertrophy measurable in about half of the patients after 6 months of treatment. The variable response to the treatment, and its inability to counteract the ataxia so far, should prompt identification and development of new molecules possibly targeting the induction of cells early antioxidant defenses.

S09. Clinical trials for Friedreich Ataxia in adults

A. Dürr;
INSERM U289 et Dép.de Génétique, Cytogénétique et Embryologie, Hôpital de la Salpêtrière, Paris, France.
No abstract received.

S10. Functions and Biogenesis of Peroxisomes and the Metabolic and Molecular Basis of Peroxisomal Disorders

J. A. Wanders, H. R. Waterham;
Academic Medical Center, University of Amsterdam, Laboratory Genetic Metabolic Diseases, Departments of Pediatrics/Emma Children's Hospital and Clinical Chemistry, Amsterdam, Netherlands.
Peroxisomal disorders (PDs) are relative newcomers in the area of genetic diseases which now comprise over 20 different disorders with Zellweger syndrome as the prototype. The PDs can be subdivided into 2 groups including 1. the peroxisome biogenesis disorders (PBDs) and 2. the single peroxisomal enzyme deficiencies. In PBD patients peroxisomes are strongly deficient resulting in a generalized loss of peroxisomal functions. Within the PBD group there is not only profound *clinical* heterogeneity ranging from Zellweger syndrome with early death to infantile Refsum disease with survival into adult life but also marked *genetic* heterogeneity with the involvement of at least 12 different genes. Most of these genes, called PEX-genes, have been identified allowing molecular diagnosis in virtually all patients. Peroxisomes catalyze a range of important metabolic functions including fatty acid beta-oxidation, etherphospholipid biosynthesis, fatty acid alpha-oxidation and glyoxylate detoxification, a.o. In recent years many peroxisomal disorders have been identified resulting from single peroxisomal enzyme deficiencies in each of these pathways with X-linked adrenoleukodystrophy as the most frequent single peroxisomal defect. This advanced knowledge has led to the development of highly reliable methods for the post- and prenatal laboratory diagnosis of patients at the metabolite, enzyme and DNA-level. Despite these many achievements much remains to be learned about the pathogenesis of peroxisomal disorders and about treatment strategies.

S 4. CNS function in man and model organisms

S11. The search for autism susceptibility genes

A. Monaco[1], IMGSAC[2], SLIC[3];
[1]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, [2]The International Molecular Genetic Study of Autism Consortium (http://www.well.ox.ac.uk/~maestrin/iat.html), United Kingdom, [3]The Specific Language Impairment Consortium (http://www.well.ox.ac.uk/monaco/dianne/index.shtml), United Kingdom.
Autism is characterised by impaired social interaction and communication, and is accompanied by repetitive and stereotyped behaviours and interests. Autism has an onset in the first three years, persists throughout life, and is associated with mental handicap and epilepsy although it can include milder, but related impairments in individuals of normal intelligence. From family and twin studies there is substantial evidence that autism has a strong yet complex genetic component. In order to identify autism susceptibility genes the IMGSAC has collected approximately 250 families with more than one child or relative affected. 83 sibling-pairs families with autism were screened for linkage using a whole genome scan and areas of increased allele sharing were genotyped with 119 markers in a further 69 sibling pair families. Four regions of linkage were identified including chromosome 2q (MLS 3.74), 7q (MLS 3.20), 16p (MLS 2.93) and 17q (MLS 2.34), three of which have been replicated by other groups. Candidate gene and association studies are now in progress in order to isolate the susceptibility genes at these loci. On chromosome 7q31, the FOXP2 gene is mutated in a severe monogenic form of speech and language impairment and encodes a transcription factor containing a polyglutamine tract and a forkhead domain. FOXP2 was tested for association and mutation in both complex language impairments and autism. No association or coding-region variants in FOXP2 were found and the gene is therefore unlikely to play a major role in autism or more common forms of language impairment.

S12. *C. elegans*: an animal model for high-throughput functional genomics

R. Baumeister;
ABI/Biochemistry, Laboratory of Molecular Neurogenetics, Ludwig-Maximilians-University, Munich, Germany.
The different genome projects have resulted in an exponential increase in sequence information available in the databases. At the same time, the number of functionally characterized genes is only increasing linearly. How can we increase the speed of functional genomics to make full use of the data mining? Model organisms have helped significantly to understand the roles of particular genes in an organism. However, the time and effort to perform even single targeted gene manipulations in mouse or *Drosophila melanogaster* is significant, and the complexity of these organisms prevents in many cases the detailed analyses of the KO consequences. The nematode *C. elegans* offers several advantages: About 60 % of the human disease genes are represented by homologues in *C. elegans*. In addition, the animals are small enough to be kept in large numbers in a format that allows mass manipulations (microtiter plates) and knock-outs of candidate genes can be obtained in a matter of 4-6 weeks. In addition, *C. elegans* is the only multicellular organism for which the development of each single cell and the entire connectivity of its nervous system are known. At the same time, the cellular diversity of the *C. elegans* nervous system is remarkable, including the same neurotransmitters, although the total number of neurons is only 302. We will focus on *C. elegans* models of genes involved in human neurodegenerative diseases, in particular on methods and technologies that can be automated in order to accelerate functional genomics.

S13. Proteolysis and Alzheimer's disease

C. Haass;
Ludwig-Maximilians-University, Adolf-Butenandt-Institute, Munich, Germany.
No abstract received.

S 5. Molecular karyotyping

S14. Molecular karyotyping and array CGH

P. Lichter;
Deutsches Krebsforschungszentrum, Abt. Organisation komplexer Genome, Heidelberg, Germany.
No abstract received.

S15. Multicolor FISH in two and three-dimensions

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For our multicolor-FISH applications we are currently using seven different fluorochromes for probe labeling on a routine basis. DAPI is used in addition for DNA-counterstaining. This multitude of different fluorochromes allows a wide range of different multicolor approaches for diagnostic applications and basic research. In 24-color karyotyping (multiplex-FISH/M-FISH) this increase in fluorochromes has several advantages including a significant reduction of probe complexity, facilitation of image analysis, and most importantly improvement of resolution. We estimate that interchromosomal rearrangements can be diagnosed if the translocated or inserted segment has a size in the range of 230 kb to 2.6 Mb. Furthermore, application of the latest software developments has allowed to simultaneously visualize and analyze multiple small region specific probes (BACs, PACs, YACs) in an automated fashion. Thus, sophisticated probe sets can now be tailored for specific purposes, e.g. a high resolution screen of all subtelomeric regions in one hybridization.In addition, multicolor FISH is now amenable to applications in interphase-FISH. Our technology of 3D-deconvolution microscopy employs an epifluorescence microscope equipped with a motorized table to collect a stack of images at defined levels in z-direction. Images are processed by deconvolution to remove out-of-focus information. Subsequently, 3D-reconstruction algorithms are applied. This approach allows the simultaneous analysis of up to 13 different region-specific-probes in lymphocyte-preparations and up to 7 different probes on thick (30 µm) tissue sections. Applications to

breast and ovarian tissue samples will be presented which revealed an unprecedented insight into heterogeneity and organization of these tumors on a single cell level.

S16. Measuring gene dosage by multiplex amplifiable probe hybridization

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Measuring the copy number of single-copy DNA segments can be used to screen chromosomal regions for deletion or duplication. Multiplex Amplifiable Probe Hybridization (MAPH) involves a combination of hybridization and PCR to measure copy number at up to 60 loci simultaneously, using standard preparations of genomic DNA. Short segments of DNA (100-500bp) are used as probes, so that high-resolution measurement of copy number can be made at (for example) individual exons of genes such as BRCA1. We have assembled and characterised a set of probes in which each of the 41 unique human subtelomeric regions is represented at least once. Using this probe set, copy number at all chromosome ends in one individual can be screened in a single gel lane. A series of positive controls has been used to demonstrate the sensitivity of the probes, and 83 normal control individuals were used to assess the frequency of polymorphic copy number with no apparent phenotypic effect. At some chromosome ends, notably XpYp, we have detected copy number polymorphisms on a scale (1-2kb) too small to detect by FISH. In screening for pathological rearrangements, the quantitative data produced by MAPH can be analysed statistically to test a null hypothesis of normal copy number, and diagnostic thresholds can be adjusted to vary the rates of false negatives and false positives. The ease with which large numbers of samples can be screened suggests the use of MAPH in primary screening of subtelomeric copy number, prior to definitive diagnosis by FISH.

S17. Multiplex PCR of Short Fluorescent Fragments: a simple, fast and reliable method for the detection of heterozygous genomic rearrangements

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The detection of heterozygous genomic deletions and duplications is technically difficult and represents a serious limitation to the complete diagnosis of many genetic diseases. We have developed Multiplex PCR of Short Fluorescent Fragments, a simple method which is based on: (i) the simultaneous amplification of several short genomic sequences using fluorescently labeled primers, (ii) the use of a limited number of cycles, (iii) the superposition of the fluorescent electropherograms and, (iv) comparison, between patients and controls, of the peaks representing the fluorescence of each amplicon. This method has already been adapted to the following genes: *MSH2*, *MLH1*, *MSH6*, *C1NH*, *SMN*, *BRCA1* et *RB1*. It has already been included into our diagnostic routine of the HNPCC syndrome, of the hereditary forms of breast and ovarian cancer and of retinoblastoma and has improved the genetic counseling of spinal muscular atrophy. Moreover, this method has allowed us to characterize precisely the boundaries of a large number of heterozygous deletions or duplications. This method is much more sensitive and rapid than the Southern blot technique commonly used, is better suited than quantitative real time PCR to the analysis of genes containing large numbers of exons, and appears to be more flexible than the *MAPH (Multiplex Amplifiable Probe Hybridization)* method, particularly because it can be rapidly adapted to large numbers of genes.

S 6. Skin deep: from skin disease to immunity

S18. Junctional Epidermolysis Bullosa: clinical and molecular features

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No abstract received.

S19. Toward Gene Therapy of Junctional Epidermolysis Bullosa (JEB)

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Cell therapy is an emerging therapeutic strategy aimed at replacing or repairing severely damaged tissue with cultured cells. Since surface epithelia experience a continuous self-renewal process during life, the success of keratinocyte-mediated cell therapy requires cultivation and transplantation of epithelial stem cells. Under the appropriate culture conditions, epithelial stem cells can be cultivated and generate autologous sheets suitable for transplantation. Cultured keratinocytes are currently used to restore severe epithelial defects.
JEB is a group of severe inherited skin diseases caused by mutations in the genes encoding laminin 5 or other components of the hemidesmosome. We show here full phenotypic correction of the adhesion properties of stratified epithelium obtained from epidermal stem cells isolated from patients suffering from laminin-5-deficient JEB, and transduced *ex vivo* with a retroviral vector expressing the ß3 chain of laminin-5. We also propose a non invasive surgical procedure that allows transplantation of cultured epidermal sheets in local anesthesia.
Thus:
- the possibility of cultivating large areas of epidermis
- the availability of surgical protocols for grafting large skin areas
- the demonstration of sustained transgene expression and stable gene correction in epidermal stem cells from JEB-patients
prompt us to propose the implementation of a phase I/II clinical trial aimed at *ex vivo* gene therapy of selected JEB patients.

S20. Anhidrotic ectodermal dysplasia with immunodeficiency is associated with genetic defects in the NF-κB pathway

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Laboratoire de Génétique Humaine des Maladies Infectieuses, Faculté de Médecine Necker-Enfants Malades, Paris, France.

The molecular basis of X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) has remained elusive until hypomorphic *NEMO* mutations were found in male EDA-ID patients from several kindreds and two patients with a related and hitherto unrecognised syndrome of EDA-ID with osteopetrosis and lymphedema (OL-EDA-ID). Mutations in the coding region are associated with EDA-ID, and stop codon mutations with OL-EDA-ID. *NEMO* encodes the regulatory subunit of the IKK complex, which is essential for NF-κB signalling. Germline loss-of-function mutations in *NEMO* have been shown to be lethal in male foetuses. In contrast, *NEMO* mutations causing OL-EDA-ID and EDA-ID are milder, as they impair but do not abolish NF-κB signalling. EDA results from impaired NF-κB signalling through the Eda receptor. Abnormal immunity in OL-EDA-ID patients results from impaired cell responses to at least Lps, IL-1ß, IL-18, TNFα and CD154. In conclusion, impaired but not abolished NF-κB signaling in humans results in two related X-linked syndromes which associate specific developmental and immunological defects. Other patients with EDA-ID still lack a genetic cause, implying that the etiological investigation of the EDA-ID syndrome should provide a molecular dissection of the NF-κB pathway.

S 7. Genome Organisation & Gene Expression

S21. Recent Duplication and the Dynamic Mutation of the Human Genome.

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It has been estimated that 5% of the human genome consists of interspersed duplicated material that has arisen over the last 30 million years of evolution. Two categories of recent duplicated segments can be distinguished: segmental duplications between non-homologous chromosomes (transchromosomal duplications) and duplications largely restricted to a particular chromosome (chromosome-specific duplications). A large proportion of these

duplications exhibits an extraordinarily high degree of sequence identity at the nucleotide level (>95%) spanning large (1-100 kb) genomic distances. Through processes of paralogous recombination, these same regions are targets for rapid evolutionary turnover among the genomes of closely related primates. The dynamic nature of these regions in terms of recurrent chromosomal structural rearrangement and their ability to generate fusion genes from juxtaposed cassettes suggests that duplicative transposition has been an important force in the evolution of our genome. Cycles of segmental duplication over periods of evolutionary time may provide the underlying mechanism for domain accretion and the increased modular complexity of the vertebrate proteome. Further, our data suggest that a small fraction of important human genes may have emerged recently through duplication processes and will not possess definitive orthologues in the genomes of model organisms. I will discuss the organization of recent segmental duplications within the human genome and their impact in terms of disease, gene innovation and rapid restructuring of the primate genome.

S22. The relationship between genome organisation and gene expression at a human telomeric region

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Weatherall Institute of Molecular Medicine, Oxford, United Kingdom.

A major challenge in the post-genomic era is to understand the relationship between genome structure and function (transcription, replication, repair and recombination). Over the past ten years it has become clear that the interaction of DNA with chromatin and the associated epigenetic modifications (DNA methylation, changes in replication timing, histone tail modification, nuclear sublocalisation) play a major role in elaborating the information encoded in DNA. However, the hierarchy of these epigenetic phenomena and the order of events in regulating nuclear processes appear complex and are largely unknown at present.
To learn more about the relationship between chromosome structure and function we have been characterising the most telomeric 300 kb region of the short arm of human chromosome 16. This is a GC-rich, Alu-dense region containing a variety of widely expressed and tissue-specific genes, including the embryonic and adult alpha-like globin genes. We have extensively characterised the structure and epigenetic modifications of this region enabling us to define a segment of the chromosome which has been maintained as a well-defined conserved syntenic region throughout evolution. This region appears to contain most, if not all, of the information required to fully regulate alpha globin gene expression in experimental assays. The critical cis-acting sequences which mediate these aspects of chromosome function are being characterised and have been shown to be dispersed throughout a region of at least 100 kb.

S23. Chromosomal Elements Conferring Epigenetic Inheritance

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In *Drosophila* the proteins of the Polycomb (PcG) and trithorax group (trxG) are necessary to maintain throughout development the differential expression patterns of developmental regulators such as the homeotic genes. PcG and trxG proteins read the activity state of their target genes, as set during the stages of determination, and “lock” the surrounding chromatin either in an active or repressed state. The control of the chromatin state is occurring through the binding of PcG and trxG proteins to common cis-elements termed Cellular Memory Module (CMM). In order to study the mode of CMM action in more detail we have established a transgene system that allows us to switch CMMs from a repressed to an activated state that is mitotically heritable. Results from this system show that transcription through a CMM is necessary for switching the activity state, which might explain the function of several non-coding RNAs transcribed close to genes potentially controlled by CMMs. In a complementing approach, we study the role of CMMs in determination and transdetermination processes in *Drosophila* imaginal discs.The molecular nature of the epigenetic mark maintaining either silencing or activation of a CMM throughout development is not known. We are investigating histone modifications as candidates for such a mark and indeed find a correlation between H3K9 methylation and PC protein localization. To determine whether this methylation plays a structural role in anchoring the PcG at CMMs, we challenged PC binding on polytene chromosomes in permeabilised salivary glands with various competitor peptides. Strikingly, only a subset of PC bound loci were competable by the H3K9 methylated peptide, suggesting that other additional histone modifications may mark the CMM.

S 8. Complex Diseases

S24. From Crohn Disease to IBD1

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Fondation Jean Dausset, Paris, France.

No abstract received.

S25. The heritability of Type 1 Diabetes: genetic bases and molecular mechanisms

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The genetic analysis of a complex trait like type 1diabetes (T1D) is complicated by many factors. Aside from *IDDM1*, the major disease superlocus located in the HLA region on chromosome 6p21, low penetrance, compounded by small individual genetic effects of the other loci create severe difficulties. Interlocus and allelic heterogeneity might further complicate the analysis. Some of the aforementioned factors could be alleviated by concentrating on an isolated and relatively homogenous population such as that from Sardinia. This island has, together with Finland, the highest incidence of T1D in the world. Children with Sardinian parents, who live in the Italian mainland, where the indigenous incidence of T1D is much lower, have the same incidence of T1D as the Sardinian children living on the island, thus supporting the role of genetic factors in the high incidence of the disease. The Sardinians represent a genetic isolate in which the substantial lack of population sub-structure reduces the risk of artifacts due to population admixture. Finally, the present time Sardinian population seems to be the result of a fixation of alleles and haplotypes, rare or absent in other European derived populations, that are particularly useful for trans-ethnic analysis to fine map the etiological variants.
Our results illustrate the advantages deriving from the genetic analysis of T1D in the Sardinian population. The relative importance in the dissection of this complex trait of population specific variables as well as of the disease related factors and of the chromosome-region specific effects will be discussed.

S26. Unravelling the genetics of thrombosis.

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Thrombosis is a complex disease. Multiple interactions between genetic and environmental factors contribute to the development of the disease. Presently, we know of six or seven genetic risk factors for venous thrombosis, that can explain about 60% of families with thrombophilia. To identify new genetic risk factors for thrombosis we design the GAIT project (Genetic Analysis of Idiopathic Thrombosphilia).
This project included 397 individuals from 21 Spanish families. In all of them we measured 43 quantitative phenotypes, and we genotyped a total of 500 highly informative genetic makers. The statistical genetic analysis has been performed using a variance component model included in the software package SOLAR.
Our results demonstrate the importance of genetic factors in determining variation in hemostasis-related phenotypes. Most importantly, over 60% of the variation in susceptibility to thrombosis is attributable to genetic factors.
From the genome-wide scan, the first undertaken to identify regions containing genes influencing variation in susceptibility to thrombotic disease and its intermediate phenotypes, we demonstrated that the G20210A mutation is functional in relation to prothrombin plasma levels and the risk of thrombosis, and the polymorphism responsible for the ABO blood group is functional in relation to plasma levels of factor VIII and factor von Willebrand .
Moreover, our analyses revealed a strong linkage between a QTL influencing FXII levels and the *FXII* gene (specifically the 46C/T FXII DNA variant; LOD = 10.21). In addition, a region on chromosome 1

showed strong evidence of linkage with free PS levels (LOD = 4.07). Another interesting result suggests that multiple loci are influencing the normal variation in APCR, and *FV* DNA variants play a relatively minor role in this normal variation in APCR. These examples as part of the current results confirm the valuable potential of this approach as a basic tool for mapping the genes of complex diseases.

S 9. Towards Treatment of Neurodegenerative Disease

S27. Repairing and protecting neurones, a dual goal for cell based therapy to the brain

M. Peschanski;
INSERM U421/IM3, Faculté de Médecine, Créteil, France.

For more than a dozen years, a major combined biological and clinical research endeavour has been dedicated to the set up of new therapeutics based upon cell and gene therapy for neurodegenerative diseases. This research essentially takes into account two determinant characteristics of all these diseases, that can be briefly summarised as follows: 1. a neurodegenerative disease is due to the loss of one or a small number of specific populations of neurones, allowing in some cases for focal intra-cerebral intervention; 2. this neuronal loss is always progressive, according however to a schedule which is quite different from one disease to another, therefore providing a potential “therapeutic time window” for protective intervention. Without a discrete knowledge of the physiopathology of the diseases, and of their specific molecular and cellular mechanisms, one can envision basically two different, and complementary therapeutic modalities for these diseases. First, one may consider substituting to the degenerated neurones, homologous cells that are capable of replacing them anatomically and functionally. This so-called “substitutive” therapeutics is essentially based, at this moment, upon the use of neural cells obtained from human foetuses following elective abortions. Second, proteins have been identified that are able to protect neurones against various experimental aggressions in animals and are, therefore, good candidates to rescue neurones affected, though not yet degenerated, during disease progression. This so-called “conservative” therapeutics is essentially attempted, at this moment, by cell or viral based transfer of a gene of interest into the brain. I will present data on Huntington’s disease.

S28. Dysfunction of wild-type huntingtin in Huntington’s Disease

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Department of Pharmacological Sciences and Center of Excellence on Neurodegenerative Diseases, University of Milano, Milan, Italy.

Huntingtin is a cytoskeletal protein which is important for neuronal survival and activity. Attention onto this protein stems from the knowledge that an expansion in the variable CAG tract in the encoding gene causes Huntington’s Disease (HD), an inherited, fatal,autosomal dominant neurodegenerative disorder characterized by selective loss of the striatal neurons (HDCRG, Cell, 1993). Evidence indicate that HD occurs through a gained toxicity of mutant huntingtin. More recently, the possibility that loss of normal huntingtin function may contribute to HD has gained considerable attention (Rigamonti, J. Neuroscience 2000; Cattaneo, Trends in Neuroscience, 2001). We indeed report that normal (but not mutant) huntingtin is able to increase the transcription of Brain Derived Neurotrophic Factor in cortex, which is then delivered to striatum via the cortico-striatal afferents and thereby acting, within striatum, as a survival factor (Zuccato, Science, 2001). Loss of this activity occurs in HD due to huntingtin’s mutation leading to striatal vulnerability. Strategies aimed at restoring normal huntingtin activities in HD may therefore be beneficial.

S29. Stem cells and functional neurogenesis in the adult brain

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Over the past decade it has become clear that stem cells in the adult mammalian brain continuously generate new neurons, predominantly in the hippocampus and olfactory bulb. Data generated in our laboratory demonstrate that ependymal cells lining the ventricular system of the brain and spinal cord function as neural stem cells in the adult CNS. Ependymal cells divide rarely to give rise to subventricular zone progenitor cells, which generate neuroblasts that migrate to the olfactory bulb. In response to a spinal cord injury, ependymal cells lining the central canal are induced to proliferate and generate migratory progeny that differentiate into astrocytes and contribute scar formation.
Recent data from our laboratory further demonstrate that these stem cells also generate neurons in unexpected regions of the brain, suggesting that adult neurogenesis is even more widespread than previously thought.
Given the expanding implications of adult neurogenesis, the central issue of whether neurons generated in the adult mammalian brain actually participate in functional synaptic circuitry has yet to be resolved. We have used virus-based transsynaptic neuronal tracing to demonstrate that neurons generated in different regions of the adult brain integrate correctly into the existing synaptic circuitry. Furthermore, we demonstrate that neurons generated in the adult brain respond to a physiological stimulus and are thus functional. Taken together, these findings may have implications for our understanding of the pathogenesis of neurodegenerative disorders and further provide a promising foundation for the development of therapeutic strategies to stimulate neurogenesis in the adult brain.

Concurrent sessions

C01. Human Dignity: In Danger of Banality?

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Increasingly, human dignity is relegated to the rank of a standard ethical principle or human right and then used as an unexplained source of blanket prohibitions (e.g. reproductive cloning, creation of embryos for research, germ line therapy ….). Once considered the source of other human rights and outside the range of any normative hierarchy, the commonplace political and legislative use of the concept of human dignity in the context of human genetics risks turning it into a banality. Drawing on the origins of the concept and tracing its legal and ethical interpretations and use since the 1948 Universal Declaration of Human Rights, we will criticize this trend and offer a different perspective that ensures its fundamental and overarching nature.

C02. Prenatal diagnosis (PND) in adult-onset disorders: survey of cases and attitudes in Portugal.

J. Sequeiros[1,2], J. Rocha[1,3], J. Pinto-Basto[1], J. Leal Loureiro[1,4], T. Coelho[1,5], A. Lopes[1,6];
[1]UnIGENe-IBMC, Univ. Porto, Portugal, [2]ICBAS - Univ. Porto, Porto, Portugal, [3]C.Gen.Clín., Porto, Portugal, [4]Hosp.S.Teotónio, Viseu, Portugal, [5]H.G.S.António, Porto, Portugal, [6]H.M.Lemos, Porto, Portugal.

Prenatal diagnosis in adult-onset diseases may be controversial due to several decades of a (physically) healthy life. We surveyed all 33 national centres offering PND, for number of requests and their attitudes regarding termination of pregnancy (TOP): 26 (79%) replied. 50 cases were ascertained (including 34 for FAP-TTRMet30, 3 HD, 3 MJD, 3 SCA2, 1 DRPLA, 3 DM). 29 amniocenteses were performed. Several were simultaneous requests for pre-symptomatic testing (PST), a very difficult situation, given time constraints and the potential for three distressful situations (PST, PND, TOP). Pregnancy was not terminated despite a ‘carrier’ result at least in 4 cases. Concerning attitudes of the ‘Commisions for TOP’, 9 answers were “unanimous”, 10 “by majority” and 5 were only the director’s; only 1/3 had a medical geneticist; 7 found the current law satisfactory, but 15 did not; 13 interpreted it as permiting TOP, while 9 did not (although 5 of these would practice it!). Only 5 centres would not accept TOP for any adult-onset disease, 19 centres would perform TOP for all diseases, 1 for all but FAP (liver transplant is now a ‘therapeutical’ option); 14 centres would not accept for PND if couple not considering TOP, but 10 would.
The non-termination of a carrier foetus will result in a pre-symptomatic test for the unborn child (precluded by ethical and/or legal reasons). PND in adult-onset diseases needs tactful counselling, special sensibility and intensive psychosocial evaluation and support. We propose a specific protocol of consults and evaluations. Current law may need redefinition.

C03. External quality assessment in genetic testing reveals patterns of errors

S. Patton[1], R. Elles[1], D. Barton[2], E. Dequeker[3], C. Mueller[4], M. Losekoot[5], B. Bakker[5], B. Rautenstrauss[6], M. Simoni[7], V. Biancalana[8], P. Vogt[9], M. Voelckel[10], D. Lohmann[11];
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Genetic testing for inherited disorders is now a routine part of laboratory medicine. Studies of the reliability of such testing have indicated significant levels of inaccuracy in laboratory reports, arising from errors in sample identification, genotyping or interpretation. These errors can have significant consequences for prenatal diagnosis and carrier testing for example. External Quality Assessment (EQA), or Proficiency testing, is one approach to quantifying these errors and can be used to raise the standards of output from laboratories. The European Molecular Genetics Quality Network (EMQN) runs EQA schemes for ten different genetic disorders. Each scheme is designed to test the ability of laboratories to interpret data in the light of clinical information supplied with a referral, and to produce a clear and accurate report. Laboratories from all the European Union countries have participated in these exercises. In 2001, the schemes evaluated 315 returns from laboratories, a 33% increase on 2000. Error rates (no. of diagnostic errors/no. of cases analysed) varied between 0.7% and 7.6%. The causes of errors include incorrect genotyping, sample swaps and incorrect interpretation of technically correct results. Examples of results and errors will be presented. The errors identified indicate a clear need for EQA to measure current standards of proficiency and encourage laboratories to raise their technical performance.

C04. Mendelian Cytogenetics Network database (MCNdb): New improved version.

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[1]Wilhelm Johannsen Centre for Functional Genome Research, Panum Institute, University of Copenhagen, Copenhagen, Denmark, [2]Dept. of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark, [3]Max-Planck-Institute for Molecular Genetics, Berlin, Germany.
Mendelian Cytogenetics Network (MCN) is a global collaboration involving >300 cytogenetic laboratories, aimed at the systematic collection of data and material from disease-associated balanced chromosomal rearrangements (DBCRs). A five year grant from the Danish National Research Foundation establishing the Wilhelm Johannsen Centre for Functional Genome Research at the University of Copenhagen has ensured continued and extended support of MCN for 2001-2006. This will include improved facilities for assisted help with retrieval of DBCRs from cytogenetic archives, improved mapping facilities in collaboration with the Max-Planck Institute for Molecular Genetics, Berlin (MPI) and systematic high resolution comparative genome hybridization (CGH) of DBCR-cases within MCN. The online version of the associated database MCNdb (http://mcndb.imbg.ku.dk) at the University of Copenhagen has been developed into a working tool for the participating laboratories: New DBCRs can be submitted online. We have improved the query interface, where clinical information and breakpoint data on more than 2100 published and unpublished DBCRs can be queried and displayed together with relevant information drawn from the OMIM morbid map, data on cytogenetic microdeletions/duplications and the murine phenotypic map, thus improving the chance to identify relevant DBCRs. YAC clones can be selected and ordered from MPI (http://www.molgen.mpg.de/~abt_rop/neurogenetics/chromosome_rearrangements.html) for FISH mapping of specific breakpoints, and the breakpoints of DBCRs are linked to the human genome map by a direct interface between MCNdb and the UCSC Human Genome Browser (http://genome.cse.ucsc.edu/goldenPath/hgTracks.html), enabling rapid identification of sequenced BAC clones for planning of FISH mapping and for direct visualization of candidate genes for specific phenotypes and diseases.

C05. A real time quantitative PCR test for trisomy 21

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[1]University Women's Hospital, Basel, Switzerland, [2]University of Basel, Basel, Switzerland.
A significant proportion of clinical genetics is involved with the analysis of gross chromosomal anomalies. In prenatal diagnosis a major concern are aneuploidies, of which Down's syndrome is the most important in live births. The detection of these gross changes is still time consuming despite modern technologies such as FISH or quantitative fluorescent PCR.
For this purpose we have developed a novel alternative using real time quantitative PCR using genetic loci in the Down's region of chromosome 21 and a control locus on chromosome 12. This locus was chosen in such a manner that it should also detect cases of Down's syndrome resulting from unbalanced Robertsonian translocations.
The assessment of the ratio of these two loci by multiplex real time PCR has shown that this technique can be used for the reliable and rapid distinction of trisomy 21 from karyotypically normal tissue (refer to Figure 1). We have now extended this test to detect trisomy 18, and it can be readily extended to examine the most common other fetal aneuploidies (13, 16, X and Y) or instances of chromosomal loss or gain. Furthermore, since it permits the rapid automatic analysis of numerous samples it is very well suited for high-throughput diagnostic settings.

C06. A telomere depletion assay for non-invasive prenatal diagnosis

M. A. Hulten, S. Dhanjal;
Warwick University, Coventry, United Kingdom.
Many different and increasingly sophisticated (and time consuming) technologies have been applied in order to separate foetal cells from maternal, but to date none of these have achieved the ultimate goal of obtaining a pure sample of all types of cells of foetal origin. We describe a novel approach for identification of foetal cells in maternal blood samples using an in vitro telomere depletion assay (TDA). We present the principle for this new assay together with preliminary experimentation substantiating its practicability and future potential. Maternal blood samples (10-20 ml) are collected in EDTA tubes. Foetal cells are firstly enriched using a Triple Density Gradient and then fixed in 3:1 methanol:acetic acid. The cells are spread on clean glass slides and aged on a hot plate (40-50°C) for 2 h. Enzymatic digestion of telomeric DNA sequences is carried out in situ, by application of 2-3 units of BAL 31 enzyme (New England Bio Labs) in 50µl buffer at 37°C for 10 mins. The enzymatic reaction is stopped by washing in 2xSSC at room temperature; the slides are then dehydrated through an ethanol series and air dried. Telomeres are identified by FISH with a pantelomeric DNA probe. Foetal and adult cell nuclei are differentiated by their respective telomere fluorescence: foetal nuclei are expected to be brightly fluorescing while adult nuclei should contain little or no telomere fluorescence. Foetal sex, identified by subsequent FISH using the Y probe, was congruent in an initial series of 12 pregnancies.

C 2. Complex diseases

C07. Mutations of the RET / GDNF / HASH1 signalling pathway in congenital central hypoventilation syndrome (CCHS, Ondine's curse)

L. de Pontual[1], V. Nepote[2], T. Attié-Bitach[1], H. Trang[2], M. Simonneau[2], M. Vekemans[1], A. Munnich[1], C. Gaultier[2], S. Lyonnet[1], **J. Amiel**[1];
[1]Necker-Enfants Malades Hospital, Paris, France, [2]R. Debré Hospital, Paris, France.
CCHS is a hitherto unexplained congenital disorder of the metabolic control of breathing. Hirschsprung disease is associated with CCHS in 25% of the cases (Haddad syndrome, HS, MIM 209880), suggesting a common defect of neural crest derived cells. The RET, GDNF and HASH1 genes were regarded as candidate genes in CCHS due to: i) their role in early neuronal differentiation, ii) the phenotype of homozygous knock-out mice, and iii) their expression in the central and peripheral nervous systems of mouse embryo.
Thirty patients were screened for RET, GDNF and HASH1 genes mutations by SSCP and direct DNA sequencing (23 CCHS and 7 HS cases). We identified a heterozygous nucleotidic variation of one

of the 3 tested genes in 6/30 patients: i) a P1039L mutation of the RET gene (HS case), ii) a recurrent GDNF gene mutation of the in 2 CCHS patients (R93W), and, iii) 2 heterozygous polyalanine tract contractions of 5/13 and 8/13 codons as well as a de novo P18T missense mutation of the HASH1 gene in 2 CCHS and 1 HS cases. These DNA variations were not found in 180 control chromosomes and concern amino acids conserved in mammalians. Although polyalanine expansions are well documented in human, contractions have not been hitherto reported. In vitro studies will investigate the putative role of these mutations on HASH1 function. Finally, these findings support the view of the involvement of genes participating to the HASH1 / RET / GDNF signalling pathway and an oligogenic inheritance of CCHS.

C08. A rare *RET* haplotype acts as risk-modifier allele in Hirschsprung disease.

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Hirschsprung disease, a common genetic disorder characterized by intestinal obstruction secondary to enteric aganglionosis, demonstrates a complex pattern of inheritance, with the *RET* proto-oncogene as a major gene and several different susceptibility loci, related to Ret-signaling pathway or other neural-crest cells developmental programs. To test whether HSCR phenotype could result by additive effect of multiple genetic defects, we investigated the role of a polymorphic *RET* variant, 2508C>T, in exon 14 of the gene, characterized by low frequency among HSCR patients and over-representation in individuals affected by sporadic medullary thyroid carcinoma. Typing several different loci across the *RET* gene, we were able to determine that not the single SNP variant, but a whole conserved haplotype displays anomalous distribution and non-random segregation in HSCR families. We provide genetic evidences about a low-penetrant protective role of this haplotype in HSCR pathogenesis and demonstrate a possible functional effect linked to *RET* mRNA expression.

C09. Meta-analysis of Celiac Disease genome screens

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Identification of genetic risk factors for multifactorial disease such as celiac disease (CD), is often carried out through systematic linkage analysis on the whole genome. Each study highlights regions of interest, which nevertheless rarely achieve the genome-wide level of significance. However, some studies tend to pinpoint the same broad area of the genome.
A meta-analysis method GSMA (Wise et al, 1999) was proposed to globally interpret full genome scan results. It has then been extended to take into account replication studies on more restricted genome regions (Wise, 2001). Briefly, this method is based on the sum of the ranks of the linkage statistics obtained for a set of chromosome bins. Four genome scans has been carried out by the partners of the European Cluster on CD, using the same statistic. Each partner also carried out replication studies on additional sample of families. GSMA was first applied to the genome scans only. Apart from the well-known risk factor in the HLA region on 6q, regions 2q, 5q, 11q and 14q were significant at the 5% level. Region 5q was pinpointed in 3 out of the 4 scans, 11q in 2 out of the 4. Region 2q contains the CTLA4/CD28 cluster whose role in CD has been suggested by other studies. Accounting for the different replication studies carried out by the European partners, strengthens the evidence for region 5q.
This study was funded by the Commission of the European Communities (QLRT-1999-00037).

C10. Genetic Dissection of the HLA Region using Haplotypes of Tasmanians with Multiple Sclerosis

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Association of the HLA class II haplotype, DRB1*1501-DQB1*0602, is the most consistently replicated finding of multiple sclerosis (MS) genetic studies. However, the high level of linkage disequilibrium (LD) in the HLA region has hindered the identification of other loci that single marker tests for association are unlikely to resolve. In order to address this issue we generated haplotypes spanning 11-12 megabases (~5cM) across the entire HLA region. The haplotypes, inferred by also genotyping relatives of 152 MS cases and 105 controls of Tasmanian ancestry, define a genomic segment from D6S276 to D6S291 including 13 microsatellite markers integrated with allele typing data for DRB1 and DQB1. Association to the DRB1*1501-DQB1*0602 haplotype was replicated. In addition, we found that the class I/extended class I region, defined by a genomic segment of ~350 kb between MOGCA and D6S265, harbours genes that independently increase risk and provide protection from MS. Log linear modelling analysis of constituent-haplotypes representing genomic regions containing class I (MOGCA-D6S265), class III (TNFa-TNFd-D6S273) and class II (DRB1-DQB1) genes indicated that having class I and class II susceptibility variants on the same haplotype provides an additive effect on risk. Moreover, we found no evidence for a disease locus in the class III region. We propose that the types of statistical approaches outlined here for the analysis of haplotypes will assist in defining the HLA's contribution to MS. More broadly, these methods provide the basis for gene localisation through the dissection of haplotypes associated with other phenotypes.

C11. Genetic epidemiology of carotid artery thickness in type 2 diabetes families of the Diabetes Heart Study

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Carotid artery intimal medial thickness (IMT) is a strong predictor of subsequent cardiovascular morbidity. The role of genetic factors in thickening of the carotid wall remains largely unknown. We hypothesize that in families with multiple members having diabetes, carotid IMT is influenced by both inherited and environmental factors. Familial aggregation of carotid IMT in the presence of type 2 diabetes was studied in 252 individuals with type 2 diabetes from 122 families enrolled in the Diabetes Heart Study. Common carotid artery IMT was measured by high-resolution B-mode ultrasonography. Other measured factors included lipid levels, body mass index, fasting glucose, hemoglobin A1c, albumin-creatinine ratio, and self-reported medical history. Heritability estimates were obtained using the variance component approach implemented in the software SOLAR. Tests of association between carotid IMT and these variables were performed using mixed model analysis that accounts for familial correlation. The sample was 89% Caucasian (11% African American), 59% female and had a mean±SD for age and duration of diabetes of 60.6±10.4 and 11.2±7.9, respectively. In a multivariate model, carotid IMT was positively associated with age (p=0.001), male gender (p=0.001), African American ethnicity (p=0.020), smoking (p=0.001), hypertension (p=0.040), and total cholesterol (p=0.019). Adjusting for age, gender and ethnicity, we estimated the heritability (h2±SE) for carotid IMT to be 0.32±0.17 (P=0.030). Further adjusting for total cholesterol, hypertension status and current smoking status yielded an estimate of 0.41±0.16 (P = 0.005). These data provide empirical evidence that sub-clinical cardiovascular disease has a significant genetic component.

C12. Comparison of strategies to detect the role of a candidate gene

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To detect the effect of a candidate gene in a multifactorial trait, one acknowledged strategy is to genotype all its known intragenic SNPs and to test the association between each SNP separately and the trait. One difficulty is that the model underlying the association of the gene to the trait can be more complex and involve a combination of intragenic SNPs. The effect of a gene might not be detected if each SNP has only small marginal effect on the trait. A second possible strategy is to test the association of the trait with the combination formed by the whole set of SNPs. Because only some of the genotyped SNPs belongs to the functional combination, the information used for this test is diluted. To overcome this drawback, a third strategy is to test the association with all the possible combinations of variable numbers of SNPs. When the functional combination is tested, the information is not diluted. The gain of power by using this strategy is balanced by the correction for multiple testing. Herein, we performed simulation studies based on genotypic data taken from three genes to compare these three strategies. Different models of correspondence between a quantitative trait and the genotype were considered. We found that the strategy that gives the best power is very model-dependent.

C 3. Molecular Genetics of Mental Retardation 1

C13. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers

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Fragile X syndrome is generally regarded as a non-progressive neurodevelopmental disorder in which premutation carriers (~55 to 200 CGG repeats; FMR1 gene) are largely unaffected. However, neurological findings of progressive action tremor, ataxia, cognitive decline, and generalized brain atrophy have recently been described in some adult males with premutation alleles. Neurohistological studies on the brains of four adult male carriers (range: 70-135 CGG repeats) who had the neurological findings reveal ubiquitin-positive, intranuclear inclusions in both neuronal and astrocytic cells, with highest frequencies (~40% of neuronal nuclei) in the hippocampus. Intranuclear inclusions were absent from Purkinje cells, although inclusions were present in a small number of neurons in the dentate nucleus and diffusely in cerebellar astrocytes. The presence of intranuclear inclusions in all brains examined to date is strongly supportive of association with the premutation alleles. Results from additional brains, including a carrier female with no neurological findings, will be presented. The mechanistic basis for the inclusions is not known. FMRP (lymphocyte) levels are generally near normal in the mid-premutation range, although FMR1 mRNA levels are elevated by 2 to 5-fold in this range. The absence of an abnormal protein product in fragile X carriers suggests that the ubiquitin-positive inclusions may reflect a general cellular (neuronal) response in which the cell's protein-degradative capacity is exceeded as a consequence of altered gene regulation. The intranuclear inclusions observed with the CAG (polyglutamine) repeat disorders (e.g., Huntington's, the SCAs) may reflect a similar (general) mechanism. These models will be discussed.

C14. The European XLMR consortium: goals, achievements and future prospects

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Up to now, molecular studies have identified 7 genes that are specifically mutated in MRX families: FMR2, OPHN1, PAK3, IL1RAPL1, TM4SF2, and ARHGEF6. Furthermore, in 2 MRXS genes mutations have been found in families with MRX: RSK2 and MECP2. On average, each MRX gene is mutated in approximately 1% of patients tested. The high heterogeneity in MRX makes it necessary to screen more than 100 probands per candidate gene. In order to have access to such a large patient panel, the European XLMR consortium was established. We collected approximately 200 well-characterized XLMR families, in which the mental retardation occurs as a trait compatible with X-linked inheritance. We exchanged DNA and cell lines of families that have been localized to the X chromosome with significant lod scores of 2 or more, smaller families with a lod score less than 2, and pairs of 3 or 2 affected male family members. In addition, mentally retarded patients with X-chromosomal rearrangements have been collected and analyzed in order to identify novel candidate genes for MRX. An overview of the collected families, as well as linkage data and mutation analysis in 7 of the MRX genes (GDI1, OPHN1, PAK3, IL1RAPL1, TM4SF2, ARHGEF6, MECP2) will be reported. The patient collection of the European XLMR consortium will be a valuable resource for the identification of novel MRX genesin the postgenome era. Extended collaborative efforts will be required to handle the massive testing of candidate genes

C15. Mutations of the human ortholog of Aristaless cause X-linked mental retardation and epilepsy.

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Mental retardation and epilepsy are common, often debilitating conditions of the human brain. Frequently associated, epilepsy and mental retardation are heterogeneous conditions. Where causes are primarily genetic, major advances have been made in the unravelling of their molecular basis. The human X-chromosome alone is estimated to harbour more than 100 genes in which mutations cause mental retardation. At least eight autosomal genes for idiopathic epilepsy have been identified, and many more for conditions where epilepsy is a feature. We have identified mutations in a novel human X-chromosome linked, Aristaless related homeobox gene (ARX), in nine X-linked families with mental retardation and epilepsy. Among these were 4 families with X-linked infantile spasms syndrome (ISSX or West syndrome; MIM 308350), 2 families with Partington syndrome (PRTS; MIM 309510, and S. Frints, unpublished), one family with X-Linked Myoclonic Epilepsy with Spasticity and Intellectual Disability (XMESID; I. Scheffer, unpublished), one MRX family (M. Partington, unpublished), and one family with syndromic XLMR (M. Partington, unpublished). Two recurrent mutations found in seven families, result in expansion of polyalanine tracts of the ARX protein. Such mutations are likely to cause protein aggregation similar to other polyalanine and polyglutamine disorders. Additionally, a missense mutation within the ARX homeodomain and a truncation mutation were identified. The ARX gene has emerged as yet another major contributor to X-chromosome linked mental retardation, similar to genes like FMR1, FMR2, ATRX, or MECP2.

C16. ARX, a novel prd-class-homeobox gene highly expressed in the telencephalon, is mutated in X-linked mental deficiency

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Investigation of a critical region in Xp22.1 involved in a non-specific X-linked mental retardation family (MRX54) enabled us to demonstrate that the disease-related gene encodes a novel homeobox protein, *Aristeless* (ARX). Further screening detected *de novo* and inherited mutations in **eleven** families affected with non specific MR, Only missense mutations and in frame duplication/insertion leading to expansion of polyamine tracts of the ARX protein were identified. DNA binding assay showed that ARX binds to palindromic sites containing two core TAAT homeodomain sites. In contrast to the other genes involved in XMR, *ARX* expression is specific to the telencephalon and ventral thalamus, and completely lacking in cerebellum. The absence of detectable brain malformation

in MR patients suggest that ARX has a specialized role in physiological processes underlying cognitive development.

C17. Identification of a new MRX gene

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X-linked mental retardation (XLMR) is an inherited condition in which the failure to develop cognitive abilities is due to mutations in one gene on the X chromosome. In the last XLMR update up to 136 conditions leading to “syndromic” or “specific” MR (MRXS) and 66 entries leading to “nonspecific” MR (MRX) are listed. For 9 of the 66 MRX entries the causative gene has been identified. The recent discovery of the contiguous gene deletion syndrome ATS-MR, characterized by Alport syndrome and mental retardation, pointed at Xq22.3 as a region containing one mental retardation gene. Comparison of the deletion extent between ATS-MR patients and patients with ATS alone allowed us to define a mental retardation critical region of about 380 kb containing four genes. We report here the identification of two point mutations, one missense and one splice site change, in the FACL4 gene in two families with nonspecific mental retardation. Analysis of enzymatic activity on lymphoblastoid cell lines from both patients demonstrated a marked reduction in activity compared to normal cells, demonstrating that both mutations are null mutations. All carrier females with either FACL4 point mutations or genomic deletions showed a completely skewed X-inactivation, suggesting a role of the gene in survival advantage. FACL4 is the tenth gene mutated in MRX and the first involving a fatty acid metabolic pathway.

C18. Mutations in the Creatine Transporter Gene (SLC6A8) in Xq28 Cause X-Linked Mental Retardation: The Important Role of Creatine Metabolism in Brain Function

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An X-linked mental retardation (XLMR) family with severe mental retardation, speech and behavioral abnormalities and seizures in affected males has been found to have a G1141C mutation in the creatine transporter gene *SLC6A8* (GenBank NM_005629). This mutation results in a glycine being replaced by an arginine (G381R) and alternative splicing since the G→C transversion occurs at the -1 position of the 5’ splice junction of intron 7. Two female relatives who are heterozygous for the SLC6A8 mutation also exhibit mild mental retardation with behavior and learning problems. Males with the mutation have highly elevated creatine in their urine and creatine uptake by fibroblasts from affected males was impaired reflecting the deficiency in creatine transport.
Based on an observed 30% excess of males in the MR population, XLMR should result in a frequency of 15% of all cases of MR. However, many surveys report less than 5% of cases resulting from XLMR. Numerous factors may be responsible for this discrepancy, a major one being few XLMR entities have an associated metabolic abnormality, which might bring them to attention. The ability to measure elevated creatine in urine or serum makes it possible to screen for *SLC6A8* deficiency in males with MR of unknown etiology. The SLC6A8 finding, in conjunction with the association of MR with the creatine biosynthesis defects (AGAT and GAMT deficiencies), clearly indicates the importance of creatine metabolism in brain function. Furthermore, all three of these errors in creatine metabolism can be detected early and may be amenable to treatment.

C19. Mutation In Neurotrypsin is Responsible For Autosomal Recessive Non-specific Mental Retardation

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Mental retardation (MR) is the most common developmental disability, affecting largely 2% of the general population. The causes of MR are diverse, but an autosomal recessive mode of inheritance may account for a significant proportion of mentally retarded individuals. The extreme genetic heterogeneity of idiopathic MR and the unavailability of large family pedigrees of nonsyndromic autosomal recessive MR has limited the use of genetic linkage to identify the disease causing genes. While a large number of X-linked mental retardation genes have been found, none of the numerous genes involved in autosomal recessive MR have been hitherto identified. Here we report the identification of the first gene involved in autosomal recessive isolated MR using homozygosity mapping in an inbred family. Genome-wide search provided evidence for linkage to a region of 13 Mb on chromosome 4q24 between markers D4S1564 and D4S402. This interval encompasses the gene PRSS12 (also known as BSSP-3) encoding a brain-specific serine protease named neurotrypsin. We identified a 4 base-pair deletion (del ACGT1391-1394) within the coding sequence that segregates with the disease. This mutation is likely a null allele as it is predicted to result in a shortened protein lacking the catalytic domain.
Our results provide the first evidence for an association between cognitive impairment and a defect in proteolytic activity of brain serine protease.

C 4. Cytogenetics

C20. Prospective screening for cytogenetic anomalies, including subtelomeric rearrangements, in children with mental retardation of unknown etiology: The Amsterdam experience

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The frequency of subtelomeric rearrangements in individuals with unexplained mental retardation (MR) is uncertain, as most studies have been retrospective and case retrieval often biased towards cases more likely to have a chromosome anomaly. After a pilot study in a group of cases selected on the basis of suspicion for a chromosome anomaly, to study the applicability of the technique (subtelomeric rearrangement in 5/30[16.7%]), a prospective study was performed in a consecutive cohort of cases with unexplained MR in an academic tertiairy center. Inclusion criteria: age<18 yrs at referral; IQ<;80; no etiologic diagnosis after complete work-up. In 266 karyotyped children, anomalies were detected in 22(8.3%;7 numerical,15 structural); of 39 cases analyzed by FISH for specific interstitial microdeletions, anomalies were found in 8. FISH analyses for subtelomeric microdeletions were performed in 184 children (44% moderate-profound MR;51% familial MR), and 1(0.5%) rearrangement was identified in a non-familial MR female with mild MR (de novo deletion 12qter). The number of probable polymorphisms(n=11;6%) was considerable.A higher total number of malformations and minor anomalies were present in the cytogenetic anomaly group(n=29) versus the group without anomalies(n=183)(p<0.05).We conclude that the frequency of cytogenetic anomalies in this prospective tertiary center study was high(1 in 10). However, the frequency of subtelomeric rearrangements was low. Possible explanations are provided. Previously proposed selection criteria for efficient subtelomeric screening were not effective in our cohort. The low yield, heavy workload, and high costs of presently available screening techniques mandates development and application of new techniques such as micro-arrays.

C21. Screening Cryptic Telomeric Rearrangements In Children With Idiopathic Mental Retardation Using An Automated Fluorescent Genotyping Strategy.

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Mental retardation is a common condition that affects largely 2 % of the general population. However, its origin remains poorly understood. Recent studies have demonstrated that cryptic unbalanced subtelomeric rearrangements contribute to a significant proportion of idiopathic syndromic mental retardation cases. Because of the limited sensitivity of routine analyses, we developed a novel strategy based upon automated fluorescent genotyping to search for non-Mendelian segregation of telomeric markers. Here we report a 10 % rate of cryptic subtelomeric rearrangements in a large series of 150 probands with severe idiopathic syndromic mental retardation and normal RHG-GTG banded karyotype. Fourteen children were found to carry deletions or duplications of one or more chromosome telomeres and two children had uniparental disomy. This study clearly demonstrates that fluorescent genotyping is a sensitive and cost-effective method that not only detects telomere rearrangements but also provides the unique opportunity to detect uniparental disomies. Our results provide evidence for the prevalence of the paternal origin of the rearrangements and emphasize the phenotypic variability of these subtelomeric rearangements. Finally we suggest giving consideration to systematic examination of subtelomeric regions in the diagnostic work-up of patients with unexplained syndromic mental retardation.

C22. Screening of dysmorphic and mentally retarded subjects with high resolution comparative genomic hybridization

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We have improved the sensitivity and specificity of comparative genomic hybridization (CGH) by using dynamic standard reference intervals instead of fixed thresholds.
In our clinical cytogenetics laboratory we use this high resolution CGH (HR-CGH) as a diagnostic tool for screening of dysmorphic and mentally retarded subjects with normal or apparently balanced G-banded karyotypes. So far we found that among 207 patients with a normal conventional karyotype 23 (11%) had small deletions or duplications, of which 18 were were interstitial and five were terminal. In addition one mosaic (47,XX+9/46,XX) was detected. Among 25 translocation carriers six deletions (24%) were detected in five patients. Four had deletions at translocation breakpoints and two had deletions elsewhere in the genome. Our data indicates the existence of a large number of interstitial abnormalities which at present can only be detected by screening the genome with HR-CGH.
By the use of an ABI 7000 we aim to characterize the abnormalities with regard to size and location. We have previously shown that HR-CGH is capable of detecting deletions as small as 3 Mb. We suspect that some of the abnormalities detected by HR-CGH in the dysmorphic and mentally retarded subjects may be this small or even smaller, thus they may be close to the theoretical detection limit of CGH which has been estimated to be about 2 Mb.
We will present results of the screening of dysmorphic and mentally retarded subjects with HR-CGH as well as results of the further characterization of certain abnormalities.

C23. Screening for telomeric rearrangements in mental retardation patients using CGH-array.

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In about 7% of idiopathic severe or moderate children mental retardation, cryptic subtelomeric rearrangements are found (Knight J.L. et al, Lancet, 1999).
Several strategies to screen for subtle telomeric rearrangements have been proposed as FISH or CGH on metaphases chromosomes, automated fluorescent genotyping and MAPH telomeric assay. To date, it remains to be demonstrated that any of these approaches is sufficiently reliable and efficient to detect low level gains such as trisomy in large scale studies settings.
To evaluate the efficiency of CGH-array to detect telomeric anomalies, we generated a chip containing the set of telomere-specific PAC clones isolated by Flint 's group. Preliminary experiments with DNA from cell lines containing constitutional known chromosomal abnormalities showed that monosomic and trisomic segments are reliably detected.
To further validate the CGH-array approach, we perform a blind test on a series of 18 DNA from patients with idiopathic mental retardation. These patients have been previously tested for telomere integrity using fluorescent genotyping at the Hopital Necker, Paris (L. Colleaux).
Among the 10 first samples analysed, abnormalities found include gains of 13 (13q telomere), gain of 18 (18p and 18q telomere), loss of 2q and gain of 3p plus several cases without aberrations. Sensibility and specificity of the CGH array will be evaluated and compared with the results previously obtained using fluorescent genotyping.

C24. Heterozygous submicroscopic inversions involving olfactory receptor-gene clusters mediate the recurrent t(4;8)(p16;p23) translocation

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The t(4;8)(p16;p23) translocation, either in the balanced or unbalanced form, has been reported in more than 14 cases. The detection of this rearrangement in routine cytogenetics is difficult. Its incidence, therefore, may be underestimated, and it could represent the second most frequent translocation in man, after the t(11q;22q). Der(4) patients have the Wolf-Hirschhorn syndrome whereas the der(8) subjects show a different spectrum of dysmorphic features. We had recently reported an inversion polymorphism of two olfactory receptors (OR)-gene clusters at 8p23 triggering de novo chromosomal rearrangements. Two OR-gene clusters are also present at 4p16. We thus investigated whether OR polymorphisms at 4p16 and 8p23 were involved in the t(4;8)(p16;p23). In one balanced and six unbalanced cases, we demonstrated that the translocation breakpoints fall at the OR-gene clusters. Heterozygous submicroscopic inversions at both the 4p-OR- and 8p-OR-gene clusters were found in all the five mothers at whose meiosis the translocation occurred. The heterozygous 4p16 inversion was found in 12% control subjects. We have previously found 8p inversion heterozygosity in 26% control subjects. In agreement with statistical expectation, 2.5% of the population was found double heterozygous. Our results suggest that rearrangements involving non-homologous chromosomes can occur as a consequence of specific genomic polymorphisms.

C25. Complex chromosome rearrangement with neocentromere formation in a fetus with IUGR.

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Neocentromeres are rare functional centromeres formed within non-centromeric chromosomal regions. We present a case of neocentromere formation detected prenatally. The karyotype was: 47,XY,del(4)(p15.3q21.1),+r(4)(p15.3q21.1).ish del(4)(D4S3360+,WHS+,D4Z1-,4qsubtel+),r(4)(D4S3360-,WHS-,D4Z1+,4qsubtel-)de novo. The fetus was missing a normal chromosome 4 but had a ring chromosome, consisting of the pericentromeric region of chromosome 4, and a deleted chromosome 4, the reciprocal product of the ring formation. In situ hybridization established that the chromosome 4 pericentromeric heterochromatin sequences were located on the ring chromosome whilst the Wolf-Hirschhorn critical

region and chromosome 4 subtelomeric regions were present on the deleted chromosome. A constriction was observed in band 4q21 of the deleted chromosome 4, indicating that a neocentromere had been formed in this band, allowing stable segregation during cell division. This chromosome abnormality was detected in cultured amniocytes from a 20 week pregnancy presenting with intrauterine growth retardation and echogenic bowel. The pregnancy resulted in intrauterine death at 33-34 weeks. On delivery, the baby weighed 640g (below the 0.4th centile) and was macerated. Anal atresia and neck webbing were evident on clinical examination. The umbilical cord had two vessels. The placenta had a small infarct. Post mortem was not performed and no further tissue was available for karyotyping.
The case will be presented and discussed in the context of current understanding of centromere structure and function and of previously reported cases of neocentromere formation.

C 5. Clinical Genetics 1

C26. Genotype and Phenotype analysis of 127 patients with Noonan Syndrome.

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Background.
Noonan Syndrome (NS) is a multiple congenital abnormality syndrome characterised by short stature, typical facial appearance, pulmonary stenosis (PS), hypertrophic cardiomyopathy (HCM), pectus deformities and cryptorchidism. It has previously been mapped to 12q24 but NS is known to be genetically heterogeneous. Mutations in the non-receptor protein tyrosine phosphatase gene PTPN11 have recently been shown to cause Noonan Syndrome in some patients. Molecular analysis may be useful to identify affected individuals, allowing more accurate genetic counselling and awareness of potential complications. Presence of the PTPN11 mutation may also predict the likelihood of specific features of NS.
Methods.
We reviewed the clinical and molecular findings of 127 patients who fulfilled the diagnostic criteria of NS. The incidence of the mutation was then compared with the presence of the major clinical features.
Results.
Mutations were identified in 52 (41%) of the group and were mostly concentrated in 2 exons. 70% of the mutation group had PS and 4% had HCM compared to 47% and 23% of the non mutation group (p=0.01 and 0.03) respectively. Frequency of short stature and pectus deformities was similar in the two groups. 76% of those with the mutation attended a mainstream school as opposed to 67% of those without and cryptorchidism was found in 84% and 68% respectively (not significant).
Conclusions.
Mutations in PTPN11 have improved the phenotypic recognition of NS and are associated with a high incidence of PS. HCM appears to be more common in other genetic forms of the syndrome.

C27. A chromosomal translocation family and mutation detection identifies MAF as a new human disease gene in ocular anterior segment development

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Chromosomal rearrangements, particularly those that are balanced, can provide a vital clue to the localisation of a gene of functional significance in the causation of the associated phenotype. There are many genes involved in the development of the anterior segment of the eye and this is reflected in the marked heterogeneity in the genetic causation of hereditary congenital cataract and anterior segment dysgenesis. We identified a three-generation family with cataract and Peters anomaly, where there was a chromosomal rearrangement in balanced and unbalanced forms with breakpoints at 16q23.2 and 5p15.3. Cloning of the 16q23.2 breakpoint identified a break through the genomic control domain of MAF, a basic region leucine zipper (bZIP) transcription factor, expressed in mammalian lens development. This breakpoint transected the common fragile site, FRA16D. Screening of other families and individuals with similar ocular phenotypes identified an R288P mutation in the highly evolutionarily conserved DNA-binding domain of MAF, in a three-generation family with cataract, microcornea and iris coloboma. The mutation co-segregated with disease in the family and was not present in 496 normal control chromosomes. The results in these two families indicate that the lens development gene, MAF, is a human disease gene in congenital cataract and anterior segment abnormality. These findings further implicate MAF in lens development and emphasise the importance of the lens in formation of the ocular anterior segment. The presence of iris coloboma in one of the families broadens the possible role of MAF in development of the anterior segment.

C28. Analysis of the phenotypic abnormalities in Lymphoedema Distichiasis Syndrome in 74 patients with FOXC2 mutations or linkage to 16q24

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Lymphoedema-Distichiasis Syndrome (LD)(OMIM 153400) is a rare, primary lymphoedema of pubertal onset, associated with distichiasis. Causative mutations have now been described in FOXC2, a forkhead transcription factor gene. Numerous clinical associations have been reported with this condition including congenital heart disease, ptosis, varicose veins, cleft palate and spinal extradural cysts. In this paper we now report clinical findings in 74 affected individuals, from 18 families and 6 isolated cases. All of these individuals were shown to have mutations in FOXC2 with the exception of one family who had two affected individuals with lymphoedema and distichiasis and linkage consistent with the16q24 locus.
The presence of lymphoedema was highly penetrant. Males had an earlier onset of lymphoedema and a significantly increased risk of complications. Lymphatic imaging confirmed the earlier suggestion that LD is associated with hyperplasia of the lymphatics rather than the hypo or aplasia seen in other forms of primary lymphoedema. Distichiasis was 94.2% penetrant, but not always symptomatic. Associated findings included ptosis (31%), congenital heart disease (6.8%) and cleft palate (4%). Other than distichiasis, the most commonly occurring anomaly was varicose veins of early onset (49%). This has not been previously reported and suggests a possible developmental role for FOXC2 in both venous and lymphatic systems. This is the first gene that has been implicated in the aetiology of varicose veins.

C29. Mutations in the *SIP1 gene* cause a distinctive dysmorphic syndrome with or without HSCR

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In 1998 we delineated a new syndrome of **characteristic facial features**- multiple congenital anomaly- mental retardation-Hirschsprung disease (HSCR) subsequently found to be caused by "new" dominant mutations in the *ZFHX1B* (or *SIP1*) gene. All the mutation positive cases from the literature (where photographs are presented) and our series show the same distinctive facial phenotype. The characteristic facial features may be used as an indicator for mutational analysis of the *ZFHX1B* gene in children. The initial cases were ascertained in the presence of HSCR as well as mental retardation but it is now apparent that this is not an invariable component of the syndrome. We illustrate this with the description of two further mutation positive cases where HSCR is not present. We

also demonstrate the evolving facial features with age in our series of fifteen mutation positive cases (ten previously unpublished) to aid other clinicians in recognition of this syndrome even in the absence of HSCR. A low recurrence risk can be given to the parents when mutation analysis is positive.
We review the genotype-phenotype correlations in the 32 published cases and our 10 new cases with *ZFHX1B* mutations or deletions. All intragenic mutations so far reported have led to a premature stop codon suggesting that the mechanism for the phenotype is haplo-insufficiency with an altered gene dosage effect. It is important to test patients with Goldberg-Shprintzen syndrome (HSCR-mental retardation-microcephaly), especially those reported with sibling recurrence, to establish whether this is a separate disorder.

C30. A classification of disorders with abnormal vertebral segmentation

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Abnormal vertebral segmentation (AVS) is an important diagnostic handle and occurs in a wide variety of syndromes, e.g. Goldenhar/facio-auriculo-vertebral spectrum, VATER/VACTERL association, trisomy 8 mosaicism and maternal diabetes. However, the use of terminology in clinical practice is not consistent and the literature is correspondingly confusing for a wide variety of radiological phenotypes. The identification of a number of murine somitogenesis genes with important roles in normal development of the axial skeleton makes it possible to approach this complex field systematically. In man, mutations in genes of the *Notch* signalling pathway are the first to enable a classification based on genotype-phenotype correlation. *Jagged1* is implicated in Alagille syndrome, which includes butterfly vertebrae, and *DLL3* in autosomal recessive spondylocostal dysostosis (SCD), in which a consistent pattern of AVS throughout the spine occurs in association with rib fusions. We propose the designation SCD type 1 for cases due to mutated *DLL3* and SCD type 2 for similar phenotypes not *DLL3*-linked. There is no definite evidence as yet that *DLL3* is implicated in autosomal dominant forms of AVS. Mutations in *ROR2* cause autosomal recessive Robinow syndrome, whilst Jarcho-Levin syndrome (spondylo*thoracic* dysostosis/dysplasia) has been mapped to 2q32.1. These are specific recognisable entities which are usually distinguishable from the many sporadic cases of AVS. We propose a system of classification based on syndromic AVS, 'pure' SCD due to disrupted somitogenesis, neural tube associated AVS, and a large group of unknowns which will become the focus of new research from murine derived candidate genes.

C31. A systematic study of limb defects in Denmark.

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We are establishing a national DNA/cell bank for congenital limb defects together with the major hospitals in Denmark, including ~100 families referred to genetic counselling at the Department of Medical Genetics, University of Copenhagen. Among these are the families reported by Thomsen (1927), Kemp and Ravn (1932) and Tage-Hansen (1938), and the 625 probands with reduction defects reported by Birch-Jensen (1949) in his doctoral thesis "Congenital Deformities of the Upper Extremities". Thomsen and Kemp/Ravn in two classical papers described two large families with autosomal dominant axial synpolydactyly (SPD) with remarkable phenotypic differences. Presently, we have extended these families to 10 and 8 generations (169 and 366 individuals), and identified the mutations as 9- and 7-residue polyalanine tract expansions of HOXD13, respectively. The developmental field affected by the shorter expansion involves digits 1-5 (including syndactyly 1-2 and duplication of the index finger) whereas the 9-residue polyalanine expansion affects digits 3-4 (synpolydactyly). The large sizes of these two families permits a detailed genotype-phenotype study, which so far confirms an enormous variability of expressivity. In one carrier of the 9-residue expansion, abnormal flexion creases were the only visible trait affected, suggesting that inclusion of this trait may increase the penetrance in other SPD families. The access to a large number of probands and families with limb defects, which often can be traced and reinvestigated over many generations, and the nation-wide public health-care system with centralized registration of all individuals in Denmark provides a unique back-bone for identifying novel limb defect genes and phenotypes.

C 6. Molecular Genetics 2

C32. Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder.

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Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by multiple clinical features that include retinal dystrophy, polydactyly, obesity, developmental delay, and renal defects. Analysis of pedigree structures led historically to the hypothesis that this disorder is inherited in an autosomal recessive fashion; subsequent positional cloning efforts identified the first three of at least seven *BBS* genes (*BBS2*, *BBS4* and *BBS6*). We have screened our cohort of 163 BBS families for mutations in these genes and, when possible by family size, have constructed haplotypes across all known *BBS* regions. We report the presence of three mutant alleles in affected individuals in several BBS pedigrees. Patients in three pedigrees have two mutations in *BBS2* and one mutation in *BBS6*, and the converse occurred in a fourth pedigree. In a fifth pedigree, the patient inherited two *BBS2* and two *BBS4* mutations. Finally, four pedigrees carried a single *BBS2* mutation but have been excluded genetically from *BBS2*, whereas another three pedigrees carry a single *BBS4* mutation but have likewise been excluded genetically from *BBS4*. We propose that BBS may not be a single-gene recessive disorder but a complex trait, possibly requiring the participation of multiple loci to manifest the phenotype. Consistent with this hypothesis, in two pedigrees segregating three *BBS* mutant alleles, we have identified unaffected individuals who carry two *BBS2* mutations but not a *BBS6* mutation. This model of disease transmission may be important in the study of genetic heterogeneity in recessive disorders and for modeling gene interactions in complex traits.

C33. A Mutation in ARH Gene and a Chromosome 13q Locus Influence Cholesterol Levels in a New Form of Digenic Recessive Familial Hypercholesterolemia

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The heterogeneity of familial hypercholesterolemia (FH) is being unravelled by the discovery of novel loci contributing to this disease. Recently; mutations in a new gene, termed LDL receptor adaptor protein have been discovered in families with recessive FH. We encountered a Syrian family in which the parents and 3 of their 6 children are apparently normal and have normal total and LDL cholesterol levels. In contrast, the other three offspring have high total and LDL cholesterol levels and large xanthomas. The extended pedigree allowed us to examine another 64 members of the family. We performed a wide-genome scan in the core family and found a significant linkage to chromosome 1 p36-p35. Surprisingly, we also

found a linkage to chromosome 13q32-q22 with the same power as on the chromosome 1 locus. We, therefore, performed an interaction analysis and found supportive evidence that mutations at both loci are indispensable to display the phenotype. We also genotyped our twin panel for the informative markers at both loci and found that both loci contribute quantitatively to both total and LDL cholesterol level. We also identified the exon-intron positions of the LDL receptor adaptor protein and sequenced the gene in our family. The mutation in our family is a transversion mutation that affects the splice-accpetor site of intron 1 converting it from AG to AC. The gene on chromosome 1 locus has been already identified. Identifying chromosome 13 locus and elucidating the mechanisms by which these two genes act will be of a major importance.

C34. Large deletion of GJB6 gene in deaf patients heterozygous for GJB2 gene : genotype and phenotype analysis

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Hearing loss is the most frequent sensorial defect. At birth, 1/1000 child presents with a severe or profound hearing loss. 60% of the prelingual hearing loss are presumed to have a genetic origin. GJB2 gene represents the major genetic form of prelingual deafness as it accounts for 40% of the congenital hearing loss. GJB2 gene has been analysed in 206 independant patients with non syndromic prelingual hearing loss. 60 of them have a biallelic mutation in GJB2 (31 homozygous 35delG). Of the 36 deaf patients heterozygous for a mutation in GJB2 (12 patients 35delG/+, one V37I/+), 13 carry a deletion in trans implicating GJB6. In all families, the molecular anomalies in GJB2 and GJB6 segregate with the hearing impairement. We have determined the phenotype of the 13 composite GJB2/GJB6 heterozygous patients and have compared it to the clinical signs presented by the patient homozygous for GJB2 mutations.

C35. Genetic and functional analysis of connexins in skin disease and deafness.

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Distinct mutations in four connexins, Cx26, Cx30.3, Cx30 and Cx31, have been found to underlie sensorineural non-syndromic hearing loss (NSHL) and/or three types of hyperproliferative epidermal disease: Vohwinkel's syndrome, Hidrotic Ectodermal Dysplasia (HED) and Erythrokeratoderma variabilis (EKV).
Using PCR based analysis with dHPLC technology, we have identified a number of new mutations (both dominant and recessive) and coding polymorphisms in these four connexin genes. These and previously identified mutations have been further characterised. GFP-tagged connexin fusion proteins have been used to study mutant connexins particularly with respect to junction assembly and channel function in keratinocytes and HeLa cells. After transfection, the wildtype GFP-Cx30 fusion protein was localised at the plasma membrane in a characteristic punctate pattern showing functional gap junctions between adjoining cells. A similar localisation was observed for the NSHL mutation Cx30Thr5Met. In contrast to the wildtype and Thr5Met, the HED associated Cx30Gly11Arg and Cx30Ala88Val fusion proteins were localised to the cytoplasm. Similar genotype-phenotype differences were observed when analysing Cx26 and Cx31 mutations.
In summary, localisation data indicate that skin disease associated mutations impair protein trafficking to the plasma membrane. In contrast, NSHL mutations are capable of forming gap junction like structures at the plasma membrane but preliminary dye transfer studies suggest defective channel activity. These data show that mutations in connexin molecules can result in distinct junctional assembly and channel properties that may account for the different effects of particular mutants on epidermal function and auditory transduction.

C36. Inherited glomuvenous malformations are caused by the combination of a germline and a somatic "second hit" mutation in the glomulin gene

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Glomuvenous malformations (GVMs), localized defects of vascular morphogenesis, are single or multiple bluish-purple lesions that occur mainly in skin. Histologically, the distended veins present smooth muscle-like "glomus cells" in the media. GVMs are usually dominantly inherited and, with the more common mucocutaneous venous malformations, they are among the most frequent lesions in centers that specialize in treatment of vascular anomalies (Vikkula et al., 1998). In another abstract submitted to this meeting, we describe the criteria for clinical differential diagnosis between common VM and GVM (Boon et al.).
Using positional cloning, we recently identified the causative gene that we named glomulin (Brouillard et al., AJHG in press). As 13 of the 14 mutations identified in 20 families cause premature stop codons, GVMs are likely to be caused by loss-of-function of glomulin. As these inherited vascular lesions are localized, we hypothesized that haploinsufficiency is not enough for the development of lesions, but a somatic second hit, leading to complete localized lack of glomulin, is needed (Knudson's double-hit hypothesis for retinoblastoma). We have now screened for somatic mutations in GVM lesions, and report on the identification of a truncating mutation that was different from the patient's inherited genetic alteration and that was not seen in genomic DNA extracted from blood. Thus, it is a de novo somatic mutation in DNA of the GVM lesion. Other tissue samples are being tested to confirm this promising finding. These data support our hypothesis that GVMs are due to complete localized loss of glomulin function. (vikkula@bchm.ucl.ac.be)

C37. Coding region mutations in three acyl-CoA dehydrogenase genes may have unforeseeable consequences due to disruption of potential splice enhancer sequences

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It is becoming clear that coding region mutations do not always exert their effect simply by changing the amino acid sequence of the encoded protein. In order to examine the frequency and mechanisms underlying missplicing caused by simple coding region mutations, we have analyzed cDNA from a large number of alleles with disease-causing mutations in either of three acyl-CoA dehydrogenase (MCAD, VLCAD and SBCAD) genes. In all three genes we have identified missense mutations that despite the fact that they are located far from the exon-intron junctions lead to exon skipping as the main molecular defect. These mutations were always located in exons with suboptimal splice sites indicating a requirement for splice enhancers. Using transfection studies in CHANG- and COS-cells with wild-type and mutant MCAD minigene constructs, we have so far reproduced the exon skipping caused by one of the missense mutations (362C>T). Computer analysis indicated that this mutation disrupts an exonic splice enhancer (ESE) consensus sequence recognized by SF2/ASF, and this was examined by mutagenesis of neighboring positions. The missplicing could be corrected by cotransfection of the splicefactor SF2/ASF, and other SR-proteins also had a correcting effect. Similarly, we are underway with minigene based analysis of SBCAD and VLCAD missense mutations that are indicated to cause exon skipping as a result of splice enhancer inactivation.
We conclude that it is not uncommon that simple coding region mutations lead to disruption of ESE sequences, which are necessary for correct splicing of exons with suboptimal splice consensus sequences.

C 7. Cancer Genetics

C38. Implication of 9p21 deletion in 1p/19q-deleted oligodendrogliomas

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Oligodendroglioma is a tumour originating from oligodendrocytes, the myelin forming cells in the central nervous system. This glioma preferentially occurs in adults. It is mostly located in cerebral hemispheres with a predilection to the frontal lobe. This lesion accounts for 5-33% of all gliomas. The wide range reported for tumour occurrence reflects inter-observer discordance in histological diagnosis. Genetic analysis of oligodendroglioma has associated 1p/19q-deletions to chemosensitivity. Recently, we and others have linked a specific histological definition to this subgroup of tumors, allowing the diagnosis to be made on histological criteria prior to any genetic analysis.
Now, we have studied 9p21 deletions, p14 and p16 methylation as well as p14 and p16 mutations in a series of 21 1p/19q-deleted oligodendrogliomas. On 8 1p/19q-deleted oligodendrogliomas, presenting angiogenesis and/or necrosis, 6 had a heterozygous and 2 a homozygous deletion of 9p21. Three of them also had methylation of p16, which in one case was associated with a p16 mutation and in another to p14 methylation. These results illustrate the implication of 9p21-deletion in angiogenesis and tumor necrosis of 1p/19q-deleted oligodendrogliomas and the putative role of p16.
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C39. Cyclin L/Ania-6a, a Gene located at 3q25, is amplified and overexpressed in a Head and Neck Cancer Cell Line.

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DNA gains or amplifications on the long arm of chromosome 3 are recurrent in solid tumors from various origins, i.e. head and neck, lung, uterine cervix and ovary. To systematically map 3q amplicons in these tumors, we designed a chromosome 3 BAC/PAC array for high resolution Comparative Genomic Hybridization (or CGH array). We demonstrated, with cell lines containing constitutional chromosome 3 abnormalities, that CGH array allows the detection of low-level DNA copy number changes. By this method, we found a narrow (less than 6 Mb) high-level amplification at 3q25.3 in a head and neck cell line, Cal 27. Further mapping of the amplification by semi-quantitative PCR showed a core amplicon of 3Mb.
We performed the transcriptional comparison of Cal 27 and Hs 677.Tg, a normal head and neck cell line, with cDNA microarrays. Among 438 genes mapped on chromosome 3, the one showing the greatest overexpression (10-fold increase) is located in the core amplicon, and encodes a protein from the cyclin family, named cyclin L/ania-6a. According to these results, we propose cyclin L/ania-6a as a new oncogene at 3q25.3. Its involvement in head and neck cancer is currently evaluated by cyclin L expression measurement in a series of primary tumors.

C40. Functional analysis of MMR gene mutations linked to hereditary non-polyposis colorectal cancer

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To date, five mismatch repair (MMR) genes, MLH1, MSH2, MSH6, MSH3, and PMS2 are known to be involved in human MMR function. Two of those, MLH1 and MSH2 are further the most common susceptibility genes in hereditary non-polyposis colorectal cancer (HNPCC), whereas MSH3 and PMS2 is not or only in few cases, respectively shown to be involved in HNPCC. Although, the ever-increasing number of mutations is reported in the MSH6 gene, the mutations are mainly linked to putative HNPCC families. Especially, the early age at onset and high microsatellite instability (MSI) in tumors, the main hallmarks in HNPCC, are not typical for MSH6 mutation carriers. Based on the less typical clinical and molecular features linked to especially MSH6 but also to some MLH1 and MSH2 mutation carriers and their cancers, there is a cause to ask what really is HNPCC syndrome. High MSI is a consequence of MMR defect in the cell and consistently, the pathogenicity of germline mutations in HNPCC is linked to malfunction of MMR. To address the question, we studied the functionality of mutated MMR proteins in an in vitro MMR assay. Our results are clinically relevant since they demonstrate that in the stable in vitro circumstances, when the amounts of the proteins are adequate for repair, many mutations found from putative HNPCC families do not affect the repair function, whereas all the tested mutations found from typical HNPCC families impart a MMR deficient phenotype on the altered polypeptide.

C41. *CHK2* 1100delC is a low penetrance breast cancer susceptibility allele in non-carriers of *BRCA1* or *BRCA2* mutations

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Mutations in the two major breast cancer predisposition genes, *BRCA1* and *BRCA2*, confer a high risk of breast and ovarian cancer but only account for a small fraction of breast cancer susceptibility. As part of a search for additional susceptibility genes, we analysed *CHK2*, a cell cycle checkpoint kinase that is implicated in DNA repair processes involving BRCA1 and p53. We show that *CHK2* 1100delC, a truncating variant that abrogates the kinase activity, has a frequency of 1.1% in healthy controls. This indicates that this variant cannot be acting as a high penetrance Li-Fraumeni predisposition allele, as previously postulated, because this syndrome is very rare. However, *CHK2* 1100delC is present in 5.1% of breast cancer cases from 718 *BRCA1/2* negative breast cancer families (p=.00000003), including 13.5% of cases from families with male breast cancer cases (p=.00015). In contrast, the variant confers no increased cancer risk in carriers of *BRCA1* or *BRCA2* mutations. This suggests that the biological mechanisms underlying the elevated risk of breast cancer in *CHK2* mutation carriers are already subverted in *BRCA1/2* mutation carriers, consistent with the participation of the encoded proteins in the same pathway. We estimate that *CHK2* 1100delC confers an approximately two-fold increased breast cancer risk in women and 10-fold risk in men, and that approximately 1% of female breast cancer incidence, 9% of male breast cancer incidence and 0.5% of the familial aggregation of the disease is attributable to *CHK2* 1100delC.

C42. Heterozygosity for the NBS founder mutation in cancer patients of Czech origin.

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The chromosomal instability disorder Nijmegen Breakage Syndrome (NBS) is associated with extreme susceptibility to lymphoid malignancies due to a defect in DNA double strand break repair. Based on the „index-test-method“ we accumulated evidence that not only the homozygous patients, but also heterozygote individuals have an increased cancer risk. In order to verify this observation in an independent study, we initiated an epidemiological investigation to estimate the frequency of the major NBS1 mutation, 657del5, in new-borns; adult blood donors; and elderly over 70 years of age in comparison to its frequency among oncological patients, both children and adults, all of Czech origin.
The incidence of NBS heterozygotes was as follows: new-borns 1:158 (4/630), adult blood donors 1:226 (4/908), elderly 1:400, children with benign tumours 1:178 and with malignant tumours 1: 174 (2/348), and adult patients with malignancies 1:293. In addition to the 13 heterozygotes for the 657del5 mutation, we found one NBS homozygote (5,5 years old) with ALL. The malignant tumours of the two heterozygous children were medulloblastoma and osteosarcoma. The adult patient suffered from a non-Hodgkin lymphoma. Clearly,

the differences in the frequency of heterozygotes among the various groups are not significant. The number of individuals studied is sufficient to rule out that the general cancer risk in NBS heterozygotes is highly increased, however this does not exclude that the risk for a subset of tumours might be increased.
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C43. BCR/ABL D-FISH should be a mandatory examination for primary diagnosis of CML

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Interferon-alpha alone or in combination with cytostatic drugs can induce major and durable cytogenetic responses in about 25% of chronic myelogenous leukaemia (CML) patients. Since these patients have a significant survival benefit, frequent follow up investigations have become clinically important. It has recently been shown by Mühlmann et al. [Gene Chromosome Canc 21; 90-100; 1998] that fluorescence in situ hybridisation (FISH) on peripheral blood samples reveals results comparable with conventional cytogenetics and reduces the number of bone marrow aspirations. We therefore have included a FISH examination in 3-monthly intervals in a prospective CML study where patients are treated with Interferon-alpha and YNK1 (oral cytarabine). For FISH we have used a highly sensitive BCR/ABL D-FISH probe. It has recently been shown by Huntly et al. [Blood, 98, 1732-1738, 2001] that with this probe deletions of the derivative chromosome 9 can be detected. These deletions provide a powerful and independent prognostic indicator in CML. From 138 of the 150 patients included in our study a total of 420 cytogenetic examinations have been performed. The majority were FISH analyses on peripheral blood samples. Additionally GTG/QFQ-banding and FISH on bone marrow samples have been performed in a subset of patients. We have detected deletions in 12,6% (16/138) of patients. A BCR/ABL sensitive D-FISH for detection of the Philadelphia chromosome and deletions of the derivative chromosome 9 should therefore be incorporated into future diagnostic strategies as well as management decisions of CML.

C 8. Clinical Genetics 2

C44. Long-Term Efficacy and Safety of Enzyme Replacement Therapy in Fabry Disease

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Background: Fabry disease, lysosomal alpha-galactosidase A (alpha-Gal A) deficiency, results from progressive accumulation of globotriaosylceramide (GL-3), particularly in the microvasculature, leading to failure of target organs, and to ischemic complications involving kidneys, heart and brain. Recombinant human alpha-GalA (r-h-alpha-GalA) enzyme replacement therapy was previously evaluated in a randomized placebo-controlled, double-blind study of 58 Fabry patients who received r-h-alpha-GalA or placebo every 2 weeks for 20 weeks. The accumulated GL-3 was cleared in renal (P<0.001), cardiac (P<0.001) and skin (P<0.001) capillary endothelial cells of the treated group (N Engl J Med 2001; 345: 9-16).
Methods: Of these patients, 55/58 (95%) have continued treatment with r-h-alpha-GalA (1 mg/kg q 2wk) for an additional 12 months in an open-label extension study.
Results: The drug was well tolerated except for minor, conservatively managed infusion reactions, the incidence of which decreased over time. The infusion reactions were associated with IgG antibody seroconversion that did not affect therapeutic effect. The IgG titers in 50% of patients decreased by at least four-fold with time. Mean infusion time decreased to 2.25 hr. Renal function remained stable throughout the study, and subset analysis of high-risk populations (age>35 and creatinine clearance<80ml) also revealed stability
at 18 months. The median percent reduction in plasma GL-3 was 100% after 12 months. GL-3 was also cleared/reduced in various renal and skin cell types. Pain scores measured by the Short Form McGill Pain questionnaire improved with therapy.
Conclusions: Long term therapy with r-h-alpha-GalA is safe, well-tolerated, reverses the disease pathology, and is clinically beneficial.

C45. Genotype-phenotype relationships in Berardinelli-Seip congenital lipodystrophy

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Generalised lipodystrophy of the Berardinelli-Seip type (BSCL) is a rare autosomal recessive human disorder with severe adverse metabolic consequences. A locus on chromosome 9 (BSCL1) has recently been identified, predominantly in African-American families. More recently, mutations in a previously undescribed gene of unknown function (BSCL2) on chromosome 11, termed seipin, have been found to be responsible for this disorder in a number of European and Middle Eastern families. We have studied the genotype/phenotype relationships in 71 affected subjects from 45 apparently unrelated pedigrees. In all subjects, hepatic dysfunction, hyperlipidaemia, diabetes mellitus and hypertrophic cardiomyopathy were significant contributors to morbidity with no clear differences in their prevalence between subjects with BSCL1, BSCL2 and those with evidence against linkage to either chromosomes 9 or 11 (designated BSCLX). BSCL1 appears to be a somewhat less severe disorder than BSCL2 with a lower incidence of premature death and a higher frequency of subjects with partial and/or delayed onset of lipodystrophy. Notably, subjects with BSCL2 had a significantly higher prevalence of intellectual impairment (36/45) than those with BSCL1 (2/22) (p<0.001) or BSCLX (0/3). In summary, generalised lipodystrophy is heterogenous in nature encompassing at least three autosomal recessive conditions. While the consequences for metabolic derangement, hepatic dysfunction and cardiac enlargement appear similar between these three groups, subjects with seipin mutations (BSCL2) appear to have a markedly higher prevalence of intellectual impairment and a higher incidence of premature death, findings which have major implications for genetic counselling

C46. Lamin A/C mutations in Charcot-Marie-Tooth disorder identify a novel laminopathy in human and mouse

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Charcot-Marie-Tooth disease (CMT) is one of the most common inherited neurological disorders, affecting 1/2500 individuals. On the basis of electrophysiologic criteria these motor and sensory peripheral neuropathies have been divided into two main groups: the demyelinating (CMT1), and the axonal types (CMT2). Homozygosity mapping, performed on 23 consanguineous algerian families including patients affected with axonal autosomal recessive Charcot-Marie-Tooth neuropathy, evidenced linkage to the 1q21.2-q21.3 region in three families. The maximal pairwise lod score, was 4.14 (q=0) at D1S2721 with the linkage interval extending from D1S305 to D1S2635. By using a candidate gene approach, homozygous LMNA (lamin A/C) founder mutation (C892T) was identified in all affected patients, causing an Arg>Cys subsitution at the highly conserved residue 298 (R298C). C892T is a founder mutation

arisen on an ancestral haplotype that we identified as covering a 1.7 cM genetic distance. This change, predicted to impair protein-protein interactions, affects all 4 isoforms derived from the gene. The ultrastructural analysis of sciatic nerves from Lmna null mice evidenced a peripheral axonopathy highly resembling to AR-CMT2, while the heterozygous knock out mice nerves were unaffected or harboured only minor changes such as slight neurofilaments accumulation. We will present genetic, histopathologic, and functional data regarding lamins involvment in peripheral nerve structure and function. The responsability of LMNA, encoding lamin A/C nuclear envelope proteins, in the pathogenesis of myopathic phenotypes (Emery-Dreifuss myopathy, Limb Girdle Muscular Distrophy and Dilated Cardiomyopathy) and, now, of autosomal recessive axonal CMT, gives new important clues to the comprehension of nerve-muscle interactions and relationships.

C47. Mutational Spectrum in the PEX7 Gene and Functional Analysis of Mutant Alleles in 78 Patients with Rhizomelic Chondrodysplasia Punctata Type 1

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Rhizomelic chondrodysplasia punctata (RCDP) is a genetic heterogeneous, autosomal recessive disorder of peroxisomal metabolism, clinically characterized by symmetrical shortening of the proximal long bones, cataracts, periarticular calcifications, multiple joint contractures, and psychomotor retardation. Most patients with RCDP have mutations in the PEX7 gene encoding peroxin 7, the cytosolic PTS2 receptor protein required for targeting a subset of enzymes to peroxisomes. These enzymes are deficient in cells of RCDP patients due to their mislocalisation to the cytoplasm.
Here, we report the mutational spectrum in the PEX7 gene of 78 patients (including 5 pairs of sibs) clinically and biochemically diagnosed with RCDP type I. We found 22 different mutations including 18 novel ones. Furthermore, we show by functional analysis that disease severity correlates with PEX7 allele activity: expression of 8 different alleles from severe RCDP patients failed to restore the targeting defect in RCDP fibroblasts, while two alleles found only in mild patients complemented the targeting defect upon overexpression. Surprisingly, one of the mild alleles comprises a duplication of nucleotides 45-52 predicted to lead to a frameshift at codon 17 and no functional peroxin 7. The ability of this allele to complement the targeting defect in RCDP cells suggests that frame restoration occurs resulting in full-length functional peroxin 7, which leads to amelioration of the predicted severe phenotype. This was confirmed in vitro by expression of the 8nt-duplication-containing sequence fused in different reading frames to the coding sequence of firefly luciferase in COS cells.

C48. Mutations in DNAH5 cause primary ciliary dyskinesia and randomization of left-right asymmetry

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Primary ciliary dyskinesia (PCD, MIM 242650) is characterized by recurrent infections of the lower and upper respiratory tract due to reduced mucociliary clearance. Other manifestations of the disesae are reduced fertility. Half of the affected off-spring exhibit a situs inversus because of randomization of left-right asymmetry. We previously localized a PCD locus to chromosome 5p, containing DNAH5 encoding a protein highly similar to the Chlamydomonas gamma- dynein heavy chain (DHC). We characterized the full-length 14-kb transcript of DNAH5. DNAH5 encodes a DHC containing a motor domain with six tandemly linked AAA (ATPases associated with diverse cellular activities) modules. Computational analysis identified three domains with strong prediction for coiled-coil domains in the c-terminal portion of the protein. Using in situ hybridization we show that the mouse ortholog Dnahc5 is expressed in respiratory epithelia, brain and the node. Sequence analysis in affected individuals of eight PCD-families with randomization of LR asymmetry identified mutations resulting in non-functional DNAH5 proteins.

C49. Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz Syndrome.

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The Smith-Lemli-Opitz Syndrome (SLOS; MIM 270400) is an autosomal recessive malformation/mental retardation (MR) syndrome which ranges in clinical severity from mild dysmorphism and moderate MR to severe congenital anomalies and intrauterine death. SLOS is caused by mutations in the delta 7 sterol-reductase gene (DHCR7; E.C. 1.3.1.21) which impair endogeneous cholesterol biosynthesis making the growing embryo dependent from exogeneous (maternal) sources of cholesterol. We here have investigated whether the apo E gene which is a major component of the cholesterol transport system in humans is a modifier of the SLOS. Common apo E genotypes and DHCR7 genotypes were determined in 103 biochemically characterized SLOS patients and in 47 of their mothers. The SLOS patients clinical severity score correlated significantly ($p = 0.009$) with the maternal but not the patients apo E genotype. In line with their effects on cholesterol levels the apo e4 allele was associated with a mild and the e2 allele with a severe SLOS phenotype ($p = 0.023$). The correlation of apo E genotype with disease severity persisted after stratification for DHCR7 genotype but disappeared when cholesterol concentrations were considered. The data suggest that apo E is involved in the transport of cholesterol from the mother to the embryo and expand the role of apo E and it´s disease associations to embryonic development and malformation.

C 9. Gene Function & Large-scale Analyses

C50. Knockout mice carrying a deletion of the Mental Retardation gene Gdi1 show impaired associative memory and altered social behavior

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Genes for non-specific mental retardation (NSMR) are thought to be responsible for development of cognitive functions. One of the recently identified genes, GD1, encodes aGdi, one of the proteins controlling the activity of the small GTPases of the Rab family in vesicle fusion and intracellular trafficking. It was suggested that, in brain, the main role of aGDI was to interact with Rab3A, the Rab protein participating in synaptic vesicle fusion and neurotransmitter release.
To establish how lack of aGDI could cause mental retardation, we generated mice carrying a deletion of Gdi1 resulting in complete loss of aGDI. The mice were viable and fertile. Histological analysis of brains revealed trilamination of the infrapyramidal mossy fibers and disorganized CA3 pyramidal cells in the hippocampus of mutants, as the only visible defect.
The Gdi1 deficient mice were normal in many tasks to assess learning capacity and emotional behavior. They were impaired in tasks requiring formation of temporal associations suggesting defects in short term memory. They also show lowered aggression and altered social behavior. Our results show that in mice, as in humans, lack of Gdi1 spares most CNS functions and preferentially impairs only a few, involved in the coordination and interaction between associative forebrain structures. Biochemical and electrophysiological analysis showed that altered behavior of the mutant mice is not dependent on Rab3A whose level and intracellular distribution are

not changed in the KO mice. It more likely depends on steps of exo/endocytosis involved in synaptic vesicle recycling.

C51. The role of different mutations found in Opitz BBB/G syndrome patients on MID1 protein function

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Opitz BBB/G syndrome (OS) is a malformation syndrome of the ventral midline. The protein encoded by the MID1 gene, which is responsible for X-linked OS, comprises five separate domains common to the RING finger protein family, i.e. the RING finger itself, two B-Boxes, a coiled-coil domain and a fibronectin III domain. The C-terminal B30.2 domain is found in a subset of these proteins. Most of the mutations identified to date cluster in this part of the MID1 protein. We have previously shown that MID1 associates with microtubules. MID1 proteins carrying a mutation in the C-terminal domain do not associate with microtubules but form cytoplasmic clots instead. Recently we found that MID1 is involved in targeting the ubiquitination machinery towards microtubule-associated PP2A by binding to its regulatory subunit a4. Binding of the a4 protein to MID1 is clearly restricted to the B-Box 1 of the protein.
Now we have identified a missense mutation in this protein domain in a patient with Opitz BBB/G syndrome. Immunoprecipitation experiments show that the binding affinity of a4 to the MID1 protein with this particular mutation is significantly reduced. Interestingly this contradicts the crystral structure-based model of Freemont et al. which proposes that the loop carrying the respective B-Box mutation is not directly involved in protein-protein interaction. In addition we found a missense mutation in the coiled-coil domain which is essential for MID1 homodimer formation and protein function. Preliminary observations indicate that a MID1 protein that carries the respective mutation in fact can no longer homodimerize.

C52. ATRX and CBP gene silencing in human neuronal precursor cells by RNA interference

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The ATR-X (α-thalassaemia/mental retardation-X linked) and Rubinstein-Taybi syndromes are two examples of mental retardation and multiple congenital malformation disorders caused by mutations in transcription regulators (ATRX and CBP, respectively). The presence of a determinate spectrum of affected tissues indicates that both the ATRX and CBP mutations modify the expression of a restricted class of genes, but these genes are as yet unidentified. A general tool to recognize the target genes of a transcriptional modulator could be the silencing of the regulator and the subsequent research of the genes that present altered expression. Since the main clinical feature of both syndromes is the mental retardation, we performed the gene silencing experiments in NT2 cells that provide a model of the human neuronal differentiation. To block ATRX and CBP gene function, we have chosen the new strategy of RNA interference. The NT2 cells were separately transfected with two small interfering RNAs, one for ATRX knock out and one for CBP blocking. The ATRX expression was specifically reduced of about 90% by the cognate siRNA, but not by the siRNA directed against CBP. Similarly, a CBP protein reduction of more than 90% was observed only when the CBP siRNA was used. The ATRX or CBP depleted NT2 cells represent an in vitro model system to study the pathogenetic mechanisms of the two syndromes by the identification of the ATRX and CBP target genes that could be involved in neuronal differentiation.

C53. Transcriptome and Transcriptosomes: Gene Expression Analysis in Human Autosomal Aneuploidy

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Autosomal trisomies are common causes of early pregnancy loss, neonatal death and multiple congenital anomalies. In postnatal life only trisomies of chromosome 21, 13 or 18 are consistently detected. Each produces a distinct clinical syndrome with considerable variability in the severity and pattern of associated malformations, which cannot be accurately predicted by the karyotype. We perform transcriptome analyses using mRNA extracted from human trisomy 21 and 13 primary amniocytes. These cells are of fetal origin and easily available following routine diagnostic testing. A commercial microarray system with ~9000 different human cDNAs was used. A subset of the microarray-derived gene expression ratios were also confirmed using the LightCycler system. Compared to normal amniocytes relatively few (0.7-3.2%) genes show substantial misregulation and these differ between the trisomies. Remarkably, when all the ratios were averaged by chromosome ("transcriptosome") only the relevant trisomic transcriptosome showed significant differences. The levels of these up-regulations were 1.14 for trisomy 21 and 1.06 for trisomy 13. The majority of altered expression is, however, secondary and scattered around the genome. We then used the bioinformatics tool PubGene to arrange expression data into potentially interacting gene networks. For example, several different matrix metalloproteinases (MMP10, MMP7, MMP1, TIMP3) are >1.7-fold upregulated in trisomy 21 whereas several insulin-like growth factor binding protein-associated genes (IGFBP4, IGFBP5, SERPINE1, EDN1, CTGF & THBS1) were >1.7 fold down-regulated. We believe that transcriptome analysis holds great potential for unravelling the molecular basis of phenotypic variation and embryopathology in chromosomal disorders.

C54. Expression Atlas of the mouse orthologues of the human chromosome 21 genes

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Down syndrome (DS), due to an extra copy of human chromosome 21 (HC21), is the most common genetic cause of mental retardation. To define where HC21 genes exert their function and identify their possible role in the DS phenotypes we performed a systematic analysis of the expression profile of 170 murine genes which represent (almost all) the orthologues of the HC21 genes. To obtain an high resolution expression pattern several complementary methods were combined: RT-PCR on a mouse cDNA panel of 12 adult tissues and 4 developmental stages; wholemount in situ of E9.5 and E10.5 embryos and section in situ of E14.5 embryos. These stages correspond to mid and late embryonic and fetal human periods, when the major organs and body regions are organized. Genes showing interesting and/or restricted expression pattern were analyzed further on appropriate sections at more developmental timepoints. 91% of the tested genes showed a clear expression pattern during murine development. In 37% of the cases expression was ubiquitous while 50% of the cDNAs showed a differential expression pattern in the embryonal tissues.
The topographical catalogue of expression of the murine orthologues of human chromosome 21 genes will be instrumental to the understanding of the pathogenesis of trisomy 21 as well as of other chromosome 21 linked disorders. Some of analyzed genes display a pattern of expression relevant to the congenital heart disease and/or to the mental retardation observed in DS. The entire data set will be made available to the scientific community via a web site.

C55. Human mitochondrial DNA diversity in the Near and Middle East and in northeastern Africa: a phylogeographic approach

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It is now well established that virtually all mtDNA variants outside Africa derive from a single sub-Saharan African mtDNA lineage L3. Descending from L3 internal nodes of the phylogenetic tree, primarily M and N, and a derivative of the latter, R, seem to give rise to all extant branches of human maternal lineages outside Africa, except the effects caused by more recent migrations. Yet it is still unclear where and when this initial diversification arose, including a

question whether largely African-specific haplogroups like M1 and U6 have arisen initially in Africa, or do they reflect selective human migrations back to Africa. We have analysed 1519 mtDNAs from Egypt, Ethiopia, Oman, Yemen, Saudi-Arabia, Jordan, Lebanon, Syria and Kuwait, as well as 1263 mtDNAs from Anatolia, South Caucasus and Iran. Detailed phylogeographic mapping of mtDNA haplogroups was carried out together with coalescence age calculations, using an additional knowledge about the European, Central Asian, Siberian and Indian populations. It became clear that individual lineage clusters within universally present basic western Eurasian mtDNA haplogroups may have very different spread in this geographically contiguous area, likely explained by deep Palaeolithic isolation, including yet poorly understood effects of the LGM to the human presence in this region. Several western Eurasian haplogroups (subgroups thereof) that are present universally, display systematically their deepest, pre-LGM coalescence ages in Egypt, Ethiopia and in Anatolia and in the South Caucasus, not in the Middle East or peninsular Arabia. Diversification in internal nodes N, R and HV is discussed.

Posters

P 1. Analysis of Disorders and Traits with Complex Inheritance

P0001. An association study of schizophrenia and the human cadherin G-type receptor Celsr1

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Cadherins play a critical role in morphogenesis and maintenance of neuronal connections in the adult brain. We examined a member of the non-classic type seven-pass transmembrane cadherins, the human homologue of Drosophila Flamingo gene - *Celsr1*. It maps to chromosome 22q13.31, a region of positive linkage results in schizophrenia and manic-depressive illness. The gene contains nine cadherin ectodomain repeats-seven EGF-like repeats, two laminin A G-type repeats coupled to a seven-pass transmembrane domain. *Celsr1* is a neural-specific gene that plays a role in early embryogenesis, cell adhesion and signal transduction.
The first exon is 3,544nt in length and encodes for the signal peptide and all nine ectodomains in the protein. We screened this exon in 24 schizophrenic patients with dHPLC followed by sequencing. Three amino-acid changes were identified and submitted to HGBASE: CTG1665GTG (L555V, SNP001026397), TCG1989TGG (S663W, SNP001026398) and CGC3375TGC (R1125C, SNP001026399). An R119G SNP from public databases (TSC0242402) was not polymorphic in this population. The three SNPs were genotyped using primer extension on ABI373 sequencers on a sample of 243 Bulgarian schizophrenic parent-offspring trios from 243 nuclear families, as well as 179 schizophrenics and 163 matched controls from UK. The three SNPs were in complete LD. There was no preferential transmission of alleles from heterozygous parents to affected offspring when analyzed with TDT. In the UK population the rare alleles were even more common in controls, the difference almost reaching statistical significance. We conclude that variations in the nine ectodomains of *Celsr1* do not increase susceptibility to schizophrenia.

Frequencies for the rare alleles (%)				
	SZ patients, BG (N = 243)	**Parents of SZ patients, BG (N = 466)**	**SZ patients, UK (N = 179)**	**Controls, UK (N = 163)**
L555V	3.9	3.1	2.8	3.7
S663W	7.7	7.2	4.5	7.7
R1125C	7.9	7.5	4.5	8.0

P0002. Longitudinal Analysis of Heteroplasmy Levels in Families with Leber Hereditary Optic Neuropathy (LHON)

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Leber hereditary optic neuropathy (LHON) is a maternally inherited ocular disease characterised by acute or subacute bilateral loss of central vision. It is associated with point mutations in the mitochondrial DNA (mtDNA). 14 % of LHON families are heteroplasmic, with a mixture of wild-type and mutant mtDNA existing within the same individual. To study whether there is any preferential selection of either the wild-type or the mutant mtDNA within individuals over time, an extensive longitudinal analysis of the segregation of the primary LHON mutations ND1/3460 and ND4/11778 was accomplished. Blood samples from 9 heteroplasmic individuals from 4 LHON families were studied over a time period of 4 to 12 years. In addition to blood samples, hair follicle and urinary tract epithelium samples of one patient were also analysed. The quantification of heteroplasmy was performed using the solid-phase minisequencing method. In one individual, the proportion of the mutant mtDNA decreased from 47 % to 42 % (p=0,034*) in 12 years. In 8 individuals, only minor changes were observed but they did not reach statistical significance. The changes did not occur in a systematic manner, suggesting absence of any simple and general selection mechanism for or against the mutant mtDNA in LHON. The various outcomes of the segregation processes can be explained by random genetic drift and/or thus far unknown nuclear control ruling the segregation of the mtDNA in each individual.

P0003. No evidence for association between a TSSP polymorphism and coeliac disease in French population.

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Celiac disease (CD) is a chronic inflammatory disease of the gut resulting from ingestion of gluten, occurring in genetically susceptible individuals. The strong genetic association of CD with HLA heterodimers is well known, but there is evidence for the involvement of additonal genetic risk factors in the HLA region. Lie et al (1999) suggested that a gene in the vicinity of D6S2223 could play a role in the pathogenesis of the disease. The TSSP gene (Thymus Specific Serine Protease), located in this region, is a good candidate because it is expected to play a role in antigen processing and presentation pathway in cortical thymic epithelial cells where it is specifically expressed. We sequenced the 12 exons of the gene and we identified 1 exonic polymorphism, a deletion of 15 bp in exon 12, at 8 bp before the stop codon (1520-1544delAGAGCCAGATTAAGG). We developed a genotyping method based on the electrophoresis on an agarose gel (2%) of PCR products and we analysed 130 French CD trios using the transmission disequilibrium test. Among 89 heterozygous parents, the deletion was transmitted in 55% of the cases (chi2=0.91, p=0.34). This result does not indicate a role of TSSP gene in the predisposition to CD. However, the identification of this deletion may be useful in the study of other auto-immune disorders.
This study was funded by the Commission of the European Communities (QLRT-1999-00037).

P0004. Study of CARD15/NOD2 gene in the susceptibility to Celiac Disease

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Celiac Disease (CD) is a gluten-sensitive enteropathy characterized by malabsorption and mucosal injury of the small bowel. The disease is associated wih both genetic and environmental risk factors. Recently, the CARD15/NOD2 gene, encoding a member of the CED4/APAF1 family of apoptosis regulators, was reported to be involved in Crohn's disease susceptibility, a chronic inflammatory disorder of the gastrointestinal tract which is associated with CD. We therefore tested the involvement of CARD15/NOD2 gene in the susceptibility to CD. Three main variants associated with Crohn's disease were genotyped in twenty five simplex celiac families for the R702W and G908R variants and in 71 simplex celiac families for the 1007fs variant. The genotyping methods were an allele-specific

PCR assay for R702W, a HhaI enzyme digestion of PCR products for G908R, and an electrophoresis on an acrylamide gel of labelled PCR products for 1007fs. Genotyping data were analysed using the transmission disequilibrium test. The frequencies of the R702W, G908R and 1007fs alleles were respectively 0.06, 0 and 0.01 in CD patients. These frequencies were not different from a control population. For R702W, only 5 parents were heterozygous and the allele was transmitted in 60% of the cases. For G908R, there were no heterozygous parents. For 1007fs, only 4 parents were heterozygous and the insertion was transmitted in 50% of the cases. Altogether, these observations do not indicate that the CARD15/NOD2 gene is a major risk factor for CD.
This study was funded by the Commission of the European Communities (QLRT-1999-00037).

P0005. Association Analysis Of The Loricrin Gene In Italian Patients With Psoriasis

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Psoriasis, (PS, OMIM 177900) is an inflammatory skin disorder affecting approximately 2% of Caucasians. PS is widely regarded as a complex trait and genome-wide scans have mapped a number of loci (PSORS 1-7) contributing to disease susceptibility. We have assigned the PSORS4 locus to chromosome 1q21, and have recently refined the susceptibility interval to 100 kb. This minimal region contains the gene for loricrin (LOR), a keratinocyte structural protein. LOR is homologous to corneosdesmosin (CDSN), an extensively investigated positional candidate for the PSORS1 locus. Interestingly, both LOR and CDSN genes show an altered expression in psoriatic lesions.
We performed a genetic analysis of the LOR gene in a sample of Italian psoriatic trios. We first re-sequenced the LOR coding region and its 5'/3' UTRs in 8 patients and identified 2 novel SNPs and a 6bp in-frame duplication. We therefore analysed these variants, as well as a previously published 12 bp duplication, in 90 trios, each including an affected offspring and both parents. We tested for association using the Transmission Disequilibrium Test and by assessing deviation from Hardy-Weinberg equilibrium (HWE). This latter test identified a significant heterozygote excess ($p = 1.8 \times 10^{-4}$) for a coding SNP in exon 2. The analysis of 40 unrelated healthy controls confirmed that the SNP is in equilibrium among unaffected, indicating that the deviation from HWE observed in patients is likely to be disease-related.
Work funded by the Italian Ministry of Health

P0006. An association between ALS and the NFH gene polymorphism

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At present the cause of amyotrophic lateral sclerosis (ALS) remains uknown. The main known ALS-causing gene is the CuZn-superoxidedismutase gene. However, ALS is complex disease and other genetic systems may be involvement in the pathogenesis of this disease.Autopsy studies have revealed aggregation and abnormal assembly of neurofilaments (NF) in the perikarya and proximal axon of motor neurons in ALS. The potential importance of NF is underscored by the observation abnormal accumulation NF in ALS patients with various mutations in CuZn-SOD. There are the tail domains of neurofilament subunits of medium and heavy molecular weight (NFM and NFH), which contains a repeated amino asid motif. In human, there are two common variants of the NFH tail, one with 45 repeats and named long (L) allele, another with 44 repeats and named short (S) allele. Previous studies have been shown that NFH tail may be involved in the pathogenesis of ALS. To investigate whether L and S allele genotype associated with ALS, we study allelic frequency in 52 patients with SALS in Moscow and control unrelated population from Russia. We have found 17 patients with LL genotype, 18 patients with LS genotype and 17 patients SS genotype, compared with 14 SS, 28 LS and 3 SS in unrelated controls. Sufficient differences in SS genotype frequency between control population and patients were observed ($X^2=9.97$, $p< 0.005$).
We conclude that SS genotype of NFH gene, probably, is associated with the pathogenesis of ALS.

P0007. Broad phenotype of child speech disorder shows strong evidence of linkage at candidate gene region 7q31

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Although the etiologies of many child speech-sound disorders are largely unknown, family and twin studies have indicated a significant genetic component for one of the more rare subtypes. A locus (SPCH1) with an autosomal dominant mode of inheritance for developmental apraxia of speech in combination with a grammatical deficit was previously localized to chromosome 7q31. We tested the hypothesis that a candidate locus on 7q31 also segregates for more common subtypes of speech disorder in 89 families (n = 196 sib pairs) ascertained through pre-school probands with moderate-to-severe speech-sound disorders of unknown origin. Our analysis included 18 markers spanning a 25.66 cM region on 7q31 positioned by using a map from the Weizmann Institute (http://bioinformatics.weizmann.ac.il). We conducted an affected sib-pair analysis using SIBPAL2 (S.A.G.E.© 4.0). Speech disorder was treated as a binary trait and adjusted for age and socio-economic status. Multi-point linkage results suggest speech disorder locus located near a 5 kb region containing markers D7S1812 ($p < 0.006$), D7S821 ($p < 0.002$), and D7S1796 ($p < 0.003$). This study provides support for the earlier hypothesis that a putative gene for speech disorders localizes to 7q31. Our studies are now being expanded to include continuous phenotypes based on a suite of metrics obtained from conversational speech samples. The next research phase will be to perform SNP analyses and radiation hybrid mapping to isolate the first putative gene for the most common type of child speech-sound disorder.
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P0008. Chronic recurrent multifocal osteomyelitis (CRMO): evidence for a susceptibility gene located on chromosome 18q21.3-18q22

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Chronic recurrent multifocal osteomyelitis (CRMO) is characterized by recurrent inflammatory lesions in the metaphyses of long bones and usually affects children and adolescents. Similarity with an autosomal recessive mouse disorder (cmo, chronic multifocal osteomyelitis) prompted us to perform a family based association study with two markers on chromosome 18q in the region homologous to the cmo localisation of the mouse. We found a significant association of CRMO with a rare allele of marker D18S60, resulting in a haplotype relative risk (HRR) of 18. This suggests the existence of a gene in this region contributing in a significant manner to the etiology of CRMO and concomitantly demonstrates evidence for a genetic basis of CRMO for the first time. This gene is not identical with RANK, which is mutated in familial expansile osteolysis (FEO), because no mutations were detected in RANK. Mutation search in RANK and the genes PIGN and KIAA1468 lead to detection of two variants (one in RANK and one in PIGN), which are in linkage disequilibrium with the rare D18S60 allele, but not independently associated with CRMO.

P0009. LDL receptor-related protein (LRP) expression pattern in male coronary patients: Inverse regulation on transcriptional and translational level

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LRP is a multifunctional cell receptor which internalizes a variety of important ligands and is therefore considered to be a candidate gene for complex diseases like atherosclerosis. **Materials and methods:** We investigated the individual LRP-mRNA and protein expression in native monocytes from 72 male probands, 36 patients with angiographically proven severe coronary atherosclerosis (age 51.7 years) and 36 healthy long-standing blood donors (age 47.3 years). The investigations on transcriptional level were carried out using a competitive RT-PCR. For specific detection of LRP protein expression we applied a macro array analysis. As a reference we used a commercially available LRP standard protein (Biomac). **Results:** We measured a significantly 1.82 fold higher LRP-mRNA expression in coronary patients in comparison to healthy controls (223ag/cell vs. 122.3ag/cell, p<0.001). However the investigation of LRP-protein expression revealed an inverse pattern: Whereas the expression of the coronary patients amounted to 1.6pg/cell the controls showed a significant higher protein expression of 6pg/cell (p<0.001). Obviously a high LRP-mRNA expression was associated with a low protein expression, and vice versa. These results suggest a complex regulatory mechanism of the LRP at transcriptional and translational level. The detected lower protein expression in coronary patients may be due to a severe unbalanced metabolism in atherosclerosis. This could lead to a diminished receptor-mediated endocytotic pathway in coronary patients which may then be compensated by increasing the mRNA expression. These findings supply evidence for the importance of the expression pattern of the receptor in the assessment of atherosclerosis development.

P0010. Power of genomic variants of TNFα and TNFß as major risk factors for coronary macroangiopathies

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The cytokines TNFα and TNFß are important cytokines in the complex signaling pathways involved in the development of atherosclerosis. **Materials and methods:** We studied three polymorphisms located in functional important regions (TNFα-promoter: G-308A, TNFß: exon2, T492C, Cys→Arg; exon3, C720A, Thr→Asp) in 198 patients with angiographically confirmed coronary diagnosis (49.7y, SD 8.5; 136 males). The patient group consists of 99 CAD-patients and 99 patients without any coronary afflictions as a control group (age- and gender-matched random probands). **Results:** The analysis of the TNFα-polymorphism (G-308A) showed an increased number of the homozygous mutation-carriers AA among CAD-patients (6 vs. 1, n.s.). Investigating the genotype frequencies of the exon2-polymorphism of TNFß we detected more mutation-carriers in the control group (0.56 vs. 0.48, n.s.). However, the examination of the TNFß-C720A-polymorphism resulted in significant differences in the two subgroups for the A-recessive model: the homozygous mutation-carriers were more often found among CAD-patients (0.18 vs. 0.07, p<0.004). Regression analysis including 5 major risk factors of CAD (smoking, hypertension, hypercholesterolemia, low LDL, Diabetes mellitus) showed a significant 3.6 times higher coronary risk for AA-carriers (95%-CI: 1.131-11.633; p<0.035). **Conclusions:** For the investigated polymorphisms in the promoter region of TNFα as well as in exon2 of TNFß no significant relation to the occurrence of CAD could be determined. However the polymorphism in exon3 of TNFß was shown to be significant associated with the risk of the development of a coronary macroangiopathy and increased the power of the investigated major clinical coronary risk factors.

P0011. Anticipation in major psychiatric disorders

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Anticipation refers to an earlier age at onset and increased severity of illness in offspring, compared to parents. It is usually caused by dynamic mutations. Anticipation has been observed in psychiatric disorders. We have collected 608 parent-offspring trios where the proband had a diagnosis of Schizophrenia (SZ), Bipolar Affective Disorder (BP) or Schizoaffective Disorder (SA). Diagnoses were made on the basis of clinical records and structured clinical interviews of probands. In 40 families (6.5%) the proband had a parent who had one of the above diagnoses. We used age at onset (AO) as the main variable for assessment of anticipation. A well-known bias operates in such studies, because psychiatric patients are less likely to have children after they get ill, so that affected parents of probands are likely to have a later AO. An additional bias operates in our sample, as all patients were quite young (all their parents were still alive). In order to reduce the bias, we looked at two subsamples: a) families where parents became ill before their child was born, and b) families where the affected offspring has children. The results were analysed with paired-samples T-test. The offspring had an earlier AO not only in the general sample, but also in a) and b) subsamples (Table 1). This study adds to the evidence that anticipation might be a true phenomenon in major psychiatric disorders, as a difference was still present after these corrections.

Table 1. Results

	BP	*SZ*	*SA*	*AO [SD] (years)*	*Mean paired difference [SD] (years)*	*Significance*
General sample, N=40						
Parents	20.0%	65.0%	15.0%	33.7 [11.2]	12.4 [12.6]	t = 6.21, p=0.000
Offspring	25.0%	55.0%	20.0%	21.4 [5.0]		
Subsample a), N=14						
Parents	-	78.6%	21.4%	22.6 [3.9]	2.8 [6.3]	t = 1.65, p=0.123
Offspring	14.3%	42.9%	42.9%	19.9 [4.8]		
Subsample b), N=12						
Parents	33.4%	50.0%	16.7%	33.9 [12.2]	12.2 [12.9]	t = 3.28, p=0.007
Offspring	33.3%	41.7%	25.0%	21.8 [5.3]		

P0012. High complexity in the genetic basis underling Factor VII deficiency in two Spanish families.

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Factor VII (FVII) is a vitamin k-dependent serine protease enzyme essential for initiating the coagulation cascade via the extrinsic pathway. Reduced FVII levels cause bleeding disease, whereas increased levels are associated with thrombosis. Some mutations have been described which modulate levels of this protein.
We have studied two different assymptomatic patients with FVII levels lower than 3% of normal values. We have sequenced the entire FVII gene (promotor, exons, introns and 3'-UTR: 15kb) to identify mutations of this gene in families.
A Gln100Arg mutation located in exon 5 was detected in one patient in the homozygote state. In this patient, we also identified a novel G to A substitution at nucleotide 3294. In another patient, we identified two different mutations: Met298Ile and Gly331Ser in exon 8. This patient was heterozygous for both of these mutations. Moreover, other FVII genetic variants that influence FVII levels, are co-segregating in these families.
Although some of these mutations have been described as closely related with bleeding disease, the presence of several putative functional genetic variants could be responsible for the high variability in phenotype observed in the family members with the same mutation. These results clearly show the complexity of FVII deficiency and the importance of a global effect of multiple quantitative trait locus (QTLs) in determining FVII levels. Further investigation should help to identify other QTLs that influence variation in FVII levels and may help to quantify the relative risk for thrombosis or bleeding disease.

P0013. Polymorphism in Serotonin Transporter Gene in Autism

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The serotonin transporter gene is a likely candidate in autistic disorder, based on efficacy of potent serotonin transporter inhibitors in reducing rituals and routines and of elevated serotonin levels been consistently found in 30%±50% of autistic patients. The aim of this study is to determine a possible association between the polymorphism in serotonin transporter gene and autism.
Materials and Methods**:** Blood samples were obtained from forty-eight individuals with autism (DSM-IV) with no associated disorders, thirty-two mothers, twenty-seven fathers (twenty-three nuclear families) and 49 normal controls.
Each family was seen for a developmental assessment, and also by a clinical geneticist for identification of eventually associated aetiology. Ages ranged between 3.6 and 41 years.
Genotyping were performed by PCR methods. Chi-square analysis was applied to the results.
Results: Within the three main possible alleles (9, 10, 12), we only observed 10 and 12. There were no differences between the frequencies distribution of autistic individuals and control group.
In autistic individuals there was no prevalence of any specific genotype. There was no difference in allele frequency between the autistic group versus mothers, fathers, or mothers and fathers.
We observed a preferential 12/12 genotype in patients with mothers carrying the same genotype. This characteristic was not observed regardind fathers genotype.
Conclusions: In this study we did no found any association of autism with the polymorphism in serotonin transporter gene .
It seems to exist a preferential 12/12 genotype in autistic individuals with mothers carrying the same genotype.

P0014. Examination of KIAA1327 on chromosome 4p15.33 for association with bipolar affective disorder

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The region on chromosome 4p15-p16 has been implicated in the aetiology of bipolar affective disorder (BP) through several linkage findings. One family collected at our department showed lod=1.96. We saturated a 9cM region on 4p with 32 microsatellites and genotyped them on sets of pooled DNA from 110 BP patients and their parents, as well as 178 patients and 184 controls. One marker was significant in the trios pool and the association was confirmed by individual genotyping. The closest gene to this marker is 170kb away: hypothetical protein KIAA1327. We screened the 17 exons, as well as 3,000bp of 5' flanking sequence which showed high homology with mouse. We used DHPLC on 15 individuals, including three key members from the linked family. We sequenced the fragments with shifts and every fragment from the key individuals.
We found five SNPs, but none of them was unique to the disease chromosome in the linked family. Four of the SNPs, including the only amino-acid change (Pro>Ile in exon 1) were genotyped in the 110 trios. None of the results reached statistical significance when analysed with TDT. The Ile allele of the Pro>Ile was transmitted 34 times and not transmitted 21 times, p=0.08. The result could not be replicated in a set of 110 trios collected in Bulgaria: 38 parents transmitted and 37 did not transmit that allele.
An interesting finding is that this SNP was in strong LD (D'=0.5) with the microsatellite that produced the original association, despite a distance of 170kb.

P0015. Confirmation of a dyslexia susceptibility gene on chromosome 1p.

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Dyslexia is a common and complex genetic trait that manifests as a specific reading disability independent of intelligence and educational opportunity. Rabin et al. (1993, Lancet 342:178-79) found suggestive evidence for linkage of dyslexia to Rh on chromosome 1p34-36. More recently, Grigorenko et al. (2001, AJMG 105:120-29) reported significant linkage to the same region, but using a quantitative definition of dyslexia. We have tested for the presence of a dyslexia gene in this region on chromosome 1p in a sample of 100 Canadian families using both qualitative and quantitative definitions of the phenotype. With the qualitative phenotype, parametric linkage analysis produced a maximum lod score of 1.7 (θ = 0.3) at D1S1597 under a recessive model with incomplete penetrance. Multipoint analyses using GENEHUNTER generated a maximum HLOD of 2.6 between markers D1S1597 and D1S3669 under the same model. Non-parametric analysis of a sub-sample of 351 sib-pairs indicated strongest linkage to D1S3669 (SIBPAL p = 0.00015). The multipoint NPL score for all the families maximized over a 15cM region spanning D1S1597 – D1S3669 – D1S199 – D1S552 (lowest p = 0.017). We also used quantitative measures of the phenotype (spelling and phonological coding) to test for linkage by the variance components method (GENEHUNTER). Using a model with QTL additive and dominance variance and polygenic additive variance, the multipoint lod score maximized over the 15cM region with a peak of 3.11 near D1S3669 for spelling. In conclusion, our study confirms and strengthens evidence for a dyslexia susceptibility gene on chromosome 1p.

P0016. Sib similarity in Danish families

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Penrose introduced sib-pair analysis in 1935: an essential feature was ascertainment for one or more affected sibs providing controls data against unrelated sib-similarity. (www.gene.ucl.ac.uk/anhumgen/).
Most recent sib-pair studies are restricted to affected sib pairs and assume the parental gametes present at ascertainment are representative of those present before fertilisation, each allele having an equal chance of both achieving fertilisation and surviving birth. Such equality is not to be expected in view of the strong preference for gametic differences in plants, with evolutionary advantages likely to be exploited in other species, and losses between conception and birth from embryonic lethals. Both would lead to excess sib-similarity at neighbouring loci. There are few data relating to sib-similarity in normal sibs.
The Danish set of normal families, occasionally supplemented by families with Mendelian disorders, was started in 1972 and has provided key information on several assignments, including CF and Batten's disease. It includes over 6000 typed individuals in 850 families with about a million genotypes.
We present estimates of the same:different ratio of paternal and maternal alleles passed from parents who were heterozygous and differed in genotype from the other parent.
Similar displays from affected sib-pairs with may be seen on www.bioch.ox.ac.uk/~jhe or www.angis.org.au/medvet

P0017. A putative molecular genetic susceptibility allele for idiopathic scoliosis

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Idiopathic scoliosis (IS) is a complex disease, with a strong genetic influence. This is supported by the familiar accumulation of the disease. It is the most frequent spine deformity of adolescence. The etiology is unknown, however difference in sex incidence was observed, the girls being affected 10 times more likely than boys. The genetic susceptibility loci of IS have not been identified so far. We accidentally were able to reveal a possible susceptibility allele for IS, in the bromodomain PHD finger transcription factor (BPTF). Two alleles with different size could be obtained with a simple PCR reaction. A 8.3 kb in homo- and heterozygotes and a 3.5 kb long in heterozygotes. No homozygotes were detected for the shorter allele. The 3.5 kb allele possibly resulted via a deletion in the last intron of BPTF and it appears more frequently in IS (p=0.29) than in control groups (p=0.16). According to a recent publication the BPTF is the

human ortholog of Drosophila Nurf301, which is the largest subunit of a chromatin remodeling NURF complex. Malfunction of BPTF in early ontogenesis, together with intense growth rate during adolescence and other environmental factors could influence the development of IS.

P0018. Deletions of 22q13 Region in Pervasive Developmental Disorders

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Pervasive Developmental Disorders (PDD) represent an heterogeneous group of behavioural deficits, including autism and atypical autism, characterized by impaired communication and social interaction, restricted interests and stereotyped behaviours. PDD affect around 1:2500 individuals within the first three years of life with a sex ratio M:F = 4:1.
A strong genetic basis has been recognized and several genomic studies, as well as cytogenetic observations, have identified at least 12 candidate loci for genes predisposing to PDD. Among these regions, 15q11-q13, 7q31 and, recently, 22q13, have been most frequently reported in association with PDD.
In order to identify the prevalence of 22q13 rearrangements (deletions) in PDD we have genotyped, by microsatellite markers analysis, 110 patients selected through a collaboration with various italian Child Neuropsychiatry centers. We detected a deletion at the D22S1169 locus in two patients (one paternally and one maternally derived) and a maternally derived deletion at the D22S1170 locus in a third patient. These patients presented with an Angelman-like phenotype, characterized by hypotonia, developmental delay and absent speech. However, they did not show epilepsy. Although the pathogenicity of these rearrangements needs to be confirmed by further studies, our results suggest that deletions within the 22q13 region might play a role in determining PDD in 2.7% of patients. Considering the marked genetic heterogeneity of PDD, this percentage appears to be relatively high.

P0019. Single Nucleotide Polymorphism Detection and Association Results - Exclusion of ITGb7 and VDR (Chromosome 12q) as Candidate Genes for Asthma

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The human genes coding for the integrin b7 (ITGb7) and the vitamin D receptor (VDR) are two of several candidate genes for asthma and related phenotypes in a promising candidate region on chromosome 12q pointed out in several genome-wide screens and candidate gene approaches. Therefore we screened the promoter region as well as all exons of the ITGb7 gene, including parts of the neighbouring introns for common polymorphisms in 32 German probands. Moreover we then tested single nucleotide polymorphisms (SNPs) for linkage/association with asthma and related traits (total serum IgE level, eosinophil cell count, peak flow and SLOPE of the dose-response curve after bronchial challenge) in a Caucasian sib-pair study (187 families with at least two affected children). We also analysed one already described SNP in the human VDR gene. We could identify three new single nucleotide polymorphisms in the ITGb7 gene. Two of them are non-coding (intron 2 and intron 6) while the SNP in exon 3 causes an substitution of the amino acid GLU to VAL.
None of the SNPs neither of the ITGb7 nor the VDR gene showed significant linkage/association with asthma or related phenotypes in this family study. From these findings we conclude that both the human ITGb7 and the VDR gene seem not to influence the pathogenesis of asthma or the expression of atopic asthmatic phenotypes as eosinophilia and changes in total IgE levels.

P0020. Significant linkage of phonological coding dyslexia to dopamine receptor type 4 (DRD4) on chromosome 11p15.5

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Phonological coding dyslexia (PCD) is a specific language disability that is independent of general intelligence and educational opportunity and is highly heritable. Because the 7-repeat allele of the dopamine D4 receptor (DRD4) exon III has been implicated in attention deficit hyperactivity disorder (ADHD), and there is known comorbidity between ADHD and dyslexia, we investigated DRD4 as a candidate gene for PCD. In our 2-point screen (FASTLINK) of the 11p15.5 region in 100 families with at least two siblings affected with PCD, we found highly suggestive evidence of linkage to markers D11S1363 (LOD=2.31, theta=0.2), DRD4-exon III repeat (LOD=2.75, theta=0.2), and HRAS (LOD 2.53, theta=0.2) . Allowing for heterogeneity (HOMOG), the HLOD for DRD4 was 3.10 with alpha=0.85. Using non-parametric affected sib-pair analysis (SIBPAL) on 254 nuclear families derived from our dataset, we also found significant linkage to DRD4 (p=0.0007) and HRAS (p=0.0011) . With multipoint linkage analysis (GENEHUNTER), we obtained a maximum between D11S1363 and DRD4 (HLOD=2.14, alpha=0.75, NPL 2.37, p=0.009). QTL analyses (SOLAR) also demonstrated a multipoint maximum near the DRD4 locus with spelling (MLOD=3.61) and phonological coding traits (MLOD=2.51). However, preliminary analysis using family-based association studies (AFBAC and ETDT) did not show significant association of PCD with the DRD4-7 repeat allele. It is possible that the DRD4-7 repeat allele is not pathogenic in dyslexia, but other mutations in the DRD4 gene not in linkage disequilibrium with the repeat are involved. Alternatively, it is possible that another gene closely linked to DRD4 in the 11p15.5 region influences susceptibility to dyslexia.

P0021. Candidate Genes Study of autoimmune Thyroid Diseases in a Large Tunisian Family

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The autoimmune thyroid diseases (AITDs) include two related disorders, Graves' disease (GD) and Hashimoto's thyroiditis (HT). The pathogenesis of the AITDs involves a complex interaction between genetic and environmental factors. Recently, five potential susceptibility loci for AITD have been mapped to chromosomes 14q31, Xq21.33, 20q11.2, 2q33 and 18q21 in different populations. In this study, we have investigated a large consanguineous Tunisian family affected with AITDs. The search for susceptibility genes in this family was undertaken by means of both linkage and association analyses. To perform linkage study, a genome screening was done using microsatellite markers. A single marker located on chromosome 2 (D2S171) showed evidence of linkage with a MMLS-c score of 3.03. However, no evidence of linkage was found for some candidate regions covering MHC, Ig VH, Cß TCR and as well as the five reported loci (**Maalej et al.** *Genes and Immunity*. **2001** Apr;2(2): 71-5). Since association analysis is more powerful than linkage analysis to detect minor susceptibility genes of complex diseases, such studies were performed on HLA loci and the CTLA-4 gene polymorphisms. Using the TDT, we have reported genetic association of GD with HLA-B*3701 (chi2= 6.12; p=0.0134), (**Bougacha et al.** *Clinical Endocrinology* **2001**, 55: 1-3) and lack of association for the intragenic CTLA-4 (AT)n and (A/G) dimorphism using the FBAT approach (p=0.406 and p= 0.466 respectively) (**Maalej et al.** *Human Immunology* **2001**, 62:1245-1250). In conclusion, our data showed linkage of **AITDs** with the **D2S171** microsatellite marker, and genetic association between **GD** and **HLA-B*3701** allele.

P0022. Gender and Strain Differences in Rhythm Parameters

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Three month old BALB/c, c57BL/6J mice and their F1 offspring were exposed, for three weeks, to 12:12 light:dark illumination. Then, over a period of 30 hours at nine equidistant times, three male and female mice of each group were sacrificed and WBC count, kidney creatine phosphokinase (CPK) and alkaline phosphatase (AP)

activities and kidney urea (U) were determined. Rhythms significance and parameters like Period, acrophase (Peak time) and amplitudes were analyzed (by Curvefit). **Results**: Comparison among rhythms revealed Gender dependent differences which in turn were also strain dependent. For example, WBC-count rhythm acrophases differed between c57BL/6J genders but not in BALB/c. The periods of AP activity rhythms differed only among c57BL/6J genders while acrophases of AP activity rhythms differed only among BALB/c gender. The amplitude of CPK activity rhythm was significantly higher in females of BALB/c then in males while the reverse was observed among c57BL/6J Genders. Gender differences were recorded also in the F1 groups. Depending on the examined variable, the rhythm differences between F1 genders may resemble those shown by one of the strains or exhibited a new difference range which didn't follow any of the gender differences (or similarity) exhibited in the parental stains. **Conclusions**: 1. Gender dependence differences exist in variable rhythms even under normal identical conditions. 2. The range of the differences among genders is strain dependent. 3. The inheritance mode suggests that each rhythm parameter is individually controlled (inherited).

P0023. Genetic mapping of malignant hyperthermia in an Israeli extended pedigree

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Malignant Hyperthermia (MH) is clinically and genetically heterogeneous disorder. At least six distinct chromosomal loci have been associated with this condition, among them RYR1 gene encoding calcium-channel accounts for about 50% of MH cases. In this study we focused on a unique Israeli family, where MH segregated along three living generations and 8 out of 10 family members were tested in a skeletal muscle contractility in-vitro assay (IVCT). A comparative genetic analysis of MH susceptible (MHS and MHE) versus normal (MHN) family members was based on polymorphic markers from two major loci linked to MH, RYR1 on chromosome 19 and DHPR on chromosome 1. Four markers within 2Mb interval spanning RYR1 gene, D19S191, D19S224, D19S228 and D19S897, were conclusive as to the linkage of MH to chromosome 19 RYR1 gene localization. Sequence analysis of RYR1 exons 11, 17, 39, 40, 45 and 46, where mutations have been previously reported in Caucasian MH pedigrees, did not reveal any variation from wild type. A potential functional polymorphism comprising non-perfect trinucleotide repeat within exon 35 was detected by sequencing and fragment analyses. Further studies on a population scale are needed to assess the population frequency and phenotypic significance of this variation. Moreover, two markers from chromosome 1, D1S2853 and D1S2683, were consistent with MH susceptible (MHS) versus MH equivocal (MHE) family members, suggesting possible involvement of DHPR gene on a refined MH phenotype.
This family might represent a unique case of complex inheritance of two genetic loci involved in predisposition to MH.

P0024. A single nucleotide polymorphism in exon 24 of the MYH7 gene may be associated with left ventricular hypertrophy in essential hypertension and with left ventricular outflow obstruction in hypertrophic cardiomyopathy

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Left ventricular hypertrophy (LVH) is known to occur as a cardiac complication of essential hypertension (EH) or as a typical structural change in dominantly inherited hypertrophic cardiomyopathy (HCM). In minority of HCM patients hypertrophy isfor unknown reasons associated with obstruction of the left ventricular outflow tract. We have investigated the association of an exon 24-SNP in the ß-myosin heavy chain gene (MYH7) with the LVH phenotype. Pyrosequencing was used for the analysis. The SNP is a silent T/C transition in codon 989. Allele frequencies in EH (n=43) and HCM patients (n=23) did not differ significantly (EH: C=0.32, T=0.68; HCM: C=0.26, T=0.74). The left ventricular mass index (in g/m^2) was calculated based on echocardiographic assessments. The following index values were obtained for CC and CT: 140 ±9.5 and 166±51 g/m^2, resp.; and for TT: 253±58 g/m^2 (significant difference by ANOVA: F=4.02, P=0.02). Outflow obstruction in HCM patients as documented by Doppler echocardiography was virtually absent in carriers of the C allele (CC or CT; n=10). However, in carriers homozygous for T (n=13) both obstruction (n=8) and non-obstruction (n=5) were observed. The difference between CC/CT carriers and TT carriers was significant as was deduced from crosstabulation tables (Pearson χ^2=8.06, P=0.0045). Conclusion: we take these results to suggest that TT homozygosity is in two different clinical conditions a marker associated with LVH in EH patients, and with obstruction in HCM patients, respectively. Conversely, the presence of the C allele may protect against LVH or left ventricular outflow obstruction.

P0025. Lack of association between a G-protein beta3-gene C825T polymorphism and attention deficit hyperactivity disorder

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G-proteins are important elements in the regulation of cellular responses where they conduct signals between receptors and effector proteins. A recently identified polymorphism C825T of a G-protein beta3 subunit (GNB3) has been shown to be associated with increased signal transduction and ion transport activity. In patients with attention deficit hyperactivity disorder (ADHD) signal transduction through several neurotransmitter systems is known to be altered. The C826T polymorphism in the GNB3 gene might contribute to the change in neurotransmitter activity. The aim of this study was to establish an association between C825T polymorphism of the GNB3 gene and ADHD. A group of 52 Slovenian children affected with ADHD was genotyped using PCR-RFLP method. The distribution of genotypes was not significantly different in ADHD patients and in healthy controls (0.403, 0.462, 0.135 vs. 0.468. 0.468, 0.064, chi square = 4.51, p = 0.1047). Also, haplotype-based haplotype relative risk analysis of 46 ADHD families (father, mother and affected child) showed no significant association between T allele and ADHD (chi square = 2.0588, p = 0.1513). Therefore, the C825T polymorphism of the GNB3 gene is unlikely to be an important genetic susceptibility factor for ADHD.

P0026. Lack of association of myo-inositol monophosphatase 2 (IMPA2) polymophisms and bipolar affective disorder

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Lithium is used as a mood-stabilizing drug in the therapy of bipolar affective disorder (BP). The IMPA2 gene codes for an enzyme in the phosphatidylinositol signalling system, which is inhibited by lithium. The IMPA2 gene is located on 18p11.2, a region implicated as a BP susceptibility locus by several linkage studies. We examined SNPs identified within this gene in 123 Bulgarian patients affected with BP and their parents. We tested 3 SNPs implicated as a disease haplotype in schizophrenia in a study by Yshikawa et al., (Mol Psych 2001, 6:202-210) and an amino-acid change (Arg148Glu) identified by Sjoholt et al., (Mol Psych 2000, 5:172-180). All four SNPs were genotyped in a multeplex assay using primer extension.
The 558C>T and 490+13-14insA (reported in the study on patients from Japan) were found to be much rarer in our population, (f of the rarer alleles = 2.4% and 0.8%, respectively). This made them unsuitable for LD mapping and we stopped genotyping after the first 30 trios. The remaining two SNPs were genotyped in 123 BP trios from Bulgaria. 58 parents were heterozygous for the 97-15G>A of whom exaclty 50% transmitted allele A to their affected offspring. The Glu allele of Arg148Glu was present in seven parents (f=1.4%).

Four of them transmitted and three did not transmit it. We genotyped a further 120 BP trios recruited in the UK. There were 8 heterozygous parents (f=1.7%) but only 2 transmitted the mutation. We cannot find support for the involvement of IMPA2 in bipolar disorder.

P0027. Autistic Spectrum Disorder: A Case Study Using High-Resolution Microarray-Based CGH

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Autism is a severe developmental disorder characterized by impairment in social interactions and in language and communication skills, and by restricted repetitive behaviors and activities.
The etiology of this disorder is poorly understood, although a variety of etiologic mechanisms have been suggested, including genetic, immunologic, infectious, neurologic and gastrointestinal abnormalities. Family and genetic studies support a strong genetic susceptibility to autistic spectrum disorders and a high incidence in family members of nonautistic PDD variants. These studies have been completed using karyotypic analysis which is limited to detecting chromosomal aberrations greater than ~ 5 Mb (<650 band resolution). With this low-resolution scan of the genome, there is a high likelihood that smaller disease associated deletion and duplication events are being missed. A modified CGH to BACs immobilized on a glass slide (instead of metaphase chromosomes) can provide a much higher resolution, potentially resolving to the size of individual BACs. We developed a human genomic array in which BACs are spaced at ~ 1 Mb intervals, on average, throughout the genome. Clinical specimens and established cell lines with a broad spectrum of known chromosomal abnormalities were tested. Test and reference genomic DNAs were differentially labeled with fluorochromes. After co-hybridization of labeled test and reference genomic DNAs, the BAC arrays were scanned and analyzed with software developed specifically for this purpose. Control hybridizations with normal male-to-male, male-to-female and female-to-female reference samples were performed and showed the expected results. This study details the results identified in the screening of over 25 autistic patients.

P0028. Variations in the vitamin-D binding protein (Gc locus) and parathyroid cell function in dialysis patients with end-stage renal disease

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Vitamin-D inhibits both PTH expression and proliferation of parathyroid cells. Patients with end-stage renal disease (ESRD) show low levels of vitamin-D due to renal dysfunction. Limiting amounts of vitamin-D do not allow a normal regulation of parathyroid function and predisposes patients to either adynamic-bone disease (ABD) or secondary hyperparathyroidism (2HP). Vitamin-D binding-protein (DBP) (*Gc locus*) is the major carrier protein for vitamin-D metabolites. Two variants at codons 416(Asp-Glu) and 420(Thr-Lys) of the exon-11 of the *Gc* gene define the three common electrophoretic variants of the DBP protein (Gc1F, Gc1S and Gc2). We have studied exon-11 alellic variants as risk factors for ABD and 2HP predisposition and parathyroid function in dialysis patients. A population of 155 patients with more than 1 year in haemodialysis were recruited and genotyped for exon-11 polymorphisms. Alelle frequencies at both codons do not differ between patients and controls. Distribution of the genotypes at both codons and the genotypes defined by the combination of the two codons were also similar in patients and controls (tables 1&2). After applying excluding criteria for treatment and time on dialysis, genotype and alelle frequencies of patients grouped as “high” and “low” PTH do not differ from those observed in control subjects. Differences for PTH levels were observed between homozygous 420LysLys and homozygous 420ThrThr patients (48.8 [95%CI:35-62] vs. 18.8 [95%CI:2.6-34.9] pmol/L, respectively, P=0,045). Variability at exon11 of the *Gc locus* seems to be not related to predisposition to ABD and 2HP. In contrast, variability at codon 420 seems to affect PTH levels.

Table 1.- Distribution of *Gc* genotypes at codons 416 and 420 in control and patients

Groups considered	codon 416 Genotypes N (%)			codon 420 genotypes N (%)		
	AspAsp	AspGlu	GluGlu	ThrThr	ThrLys	LysLys
Controls (N=139)	23(16)	83 (60)	33 (24)	65 (47)	61 (44)	13 (9)
Dialysis Patients (N=155)	31(20)	72 (47)	52 (33)	80 (51)	57 (37)	18 (12)
High PTH Group (N=35)#	4(11)	18 (52)	13 (37)	21 (60)	12 (34)	2 (6)
Low PTH Group (N=39)#	11 (28)	19 (49)	9 (23)	18 (46)	14 (36)	7 (18)

#Serum PTH levels in “high” PTH group were higher than 60 pmol/L and in “low” PTH group were lower than 12 pmol/L

Table 2.- Distribution of Gc genotypes and alelles defined by polymorphisms at the two codons

Groups considered	Gc Genotypes, N (%)						Gc Alelles , N (%)*		
	FF	SS	22	FS	F2	S2	F	S	2
Controls (N= 139)	3 (2)	33 (24)	13 (9)	29 (21))	7 (5)	54 (39)	42 (15)	149 (54)	87 (31)
Dialysis Patients (N= 155)	1 (1)	52 (34)	18 (12)	27 (17)	12 (8)	45 (29)	41 (13)	176 (57)	93 (30)
High PTH group (N= 35)#	0 (0)	13 (37)	2 (6)	8 (23)	2 (6)	10 (29)	10 (14)	44 (63)	16 (23)
Low PTH group (N= 39)#	1 (3)	9 (23)	7 (18)	8 (21)	3 (8)	11 (28)	13 (17)	37 (47)	28 (36)

* F = haplotype 416Asp-420Thr, S = haplotype 416Glu-420Thr and 2 = haplotype 416Asp-420Lys
#Serum PTH levels in “high” PTH group were higher than 60 pmol/L and in “low” PTH group were lower than 12 pmol/L

P0029. Lack of evidence for association of the endothelial nitric oxide synthase gene polymorphism in intron 4 and progression to end stage renal disease in Greek population

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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 nitric oxide synthase (ecNOS) gene has been found to affect 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. We studied the association of this polymorphism in a Greek population of patients with end stage renal disease (ESRD) by studying the genotypes of 108 ESRD patients and 105 healthy subjects. The frequencies of aa, ab, bb were 0.69, 0.28, 0.03 in the control group and 0.70, 0.26, 0.04 in the patient group. The dada between the two groups were analyzed by chi-square test. Our results from controls show that the frequencies of these three genotypes in the Greek population are similar to those observed in some other Caucasian populations. But the results from the patient group showed that the frequency of aa genotype in the patient population was not significantly different than in the control group. This work indicate that ecNOS4 polymorphism do not show any association with the development of end stage renal disease in the studied European population.

P0030. SNP Scanning in Pooled DNA – A Chance for Rapid Case-Control-Studies

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SNP Scanning in Pooled DNA - A Chance for Rapid Case-Control-Studies

Chromosome 6p21-22 is a commonly discussed region for candidate genes concerning autoimmune diseases. One possibility to find the responsible genes is to compare SNP frequencies in case-control-studies.
In a "proof of principle' approach we tested 550 SNPs in a region of 20 Mbp on chromosome 6p. All SNPs were selected from public available databases (NCBI dbSNP, TSC Consortium). SNP allele frequencies were determined by a previously validated MALDI-TOF MS based pooling approach.
Our first sample comprised 288 individuals from the Southern German population. Only SNPs with a frequency between 5% and 50% were tested. The estimated frequencies f were distributed as follows: 7,8% of all SNPs (f<10%), 21,4% (10%<f<20%), 22,4% (20%<f<30%), 26,3% (30%<f<40%) and 22% (40%<f<50%).
In a second sample of 122 German/Swedish DNAs the same 550 SNPs were tested and differences between SNP frequencies were calculated. The mean difference was 5,4%. Only 5% of all tested SNPs differed more than 15% in both samples.
The distribution of the mean allele difference will allow the assignment of possible disease associated loci. Based on this experience, we would suggest that SNP frequency differences between two samples of a case-control-study should not exceed 5-7%. This may be a strict threshold but seems practicable as large study sizes are common practice in studies of complex diseases.

P0031. Immune response to Hepatitis B and A vaccination - estimating heritability and assessing different sources of variation in twins

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The immune system is highly influenced by genetic and environmental factors. Prototypic genetic markers are the different alleles and loci of the HLA system. We estimated heritability and different sources of variation (genetic, environmental) of the immune response to Hepatitis B and A vaccination and assessed its association with several HLA-DRB1*alleles.
Methods: 96 monozygotic (MZ) and 95 dizygotic (DZ) antibody negative twin pairs were vaccinated with a combined HAV/HBsAg vaccine. Anti-HAV and anti-HBs were measured 4 weeks after the last vaccination. All twins were typed for HLA-DRB1*alleles using sequence specific PCR amplification.
Heritability was estimated based on intrapair variances. Generalized estimating equations were calculated to assess the impact of BMI, age, gender, smoking , alcohol, and HLA-DRB1*alleles. A sex limitation structural equation model was fitted to evaluate different sources of variation.
Results: HBsAg vaccine response was weaker with increasing age, body mass index and among male subjects. Several HLA-DRB1*alleles showed a positive correlation with the response. Heritability was 0.61. A model of additive genetic and environmental components best explained the observed variances. Anti-HAV response was weaker among male subjects and heritability was lower (0.35).
Conclusion: Our data suggest a major genetic contribution to the immune response to the Hepatitis B, and a minor one to the Hepatitis A vaccination. The appropriate statistical assessment of the contribution of the HLA-DRB1*alleles will be discussed.

P0032. Family based association and linkage analysis of the CD14 gene with allergic asthma in Italian familes

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The receptor for bacterial lipopolysaccharides (CD14) appears to be involved in APC-mediated Th1/Th2 cell differentiation. A polymorphism in the flanking region of the gene has been recently described to be associated with circulating soluble CD14 levels and with total serum IgE. The CD14 gene maps on chromosome 5q31, a region that has been linked to asthma and atopic responses.
We investigated whether 1 polymorphism located in the promoter of the CD14 gene (-159C/T) was associated to allergic asthma or intermediate phenotypes such as skin prick test positivity to common allergens (SPT), bronchial hyperresponsiveness to methacholine challenge (BHR), and total serum elevated IgE (IgE), in a sample of 182 asthmatic families.
Non parametric linkage analysis in affected sib-pairs did not reveal any significant result.
The transmission disequilibrium test revealed a positive association of the polymorphism with asthma (-159C p:0.0005), SPT (-159C p: 0.013), BHR (-159C p:0.0001), IgE (-159C p:0.0.009).
A multivariate analysis performed on family founder members only, did not show any significant association between the gene polymorphisms and any of the phenotypes investigated, suggesting that the CD14 gene might be an allergy susceptibility factor during childhood.
In conclusion, we confirmed the association of polymorphism -159C/T in CD14 gene with allergic asthma phenotypes in an Italian population.

P0034. CTLA-4 gene polymorphism in coeliac patients stratified by the presence of the HLA-DQ2 heterodimer

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Coeliac disease (CD) is a gluten sensitive enteropathy with multifactorial aetiology. Susceptibility to CD is strongly associated with particular HLA class II alleles, while genetic factors other than HLA remain to be determined. The cytotoxic T-lymphocyte antigen 4 (CTLA-4), a downregulator of T cell activation, has been reported both in linkage and association with CD in French and Scandinavian populations. We performed case-control and family-based association studies to investigate if the polymorphism at position 49 of the CTLA-4 exon 1 was associated with the development of CD in the Italian population. The +49 A/G dimorphism was analysed in 195 CD patients, 318 relatives and 144 ethnically matched controls by PCR-RFLP method. The A allele frequency resulted increased in patients compared with healthy controls (75.6% vs. 65.6%; $p=2.9x10^{-3}$), mostly in the homozygous form (57% vs. 45.8%; $p=2.8x10^{-2}$). The segregation analysis showed a preferential transmission of the A allele to the probands (61%; $\chi^2_{TDT}=4.15$). In order to test for an interaction between CTLA-4 and HLA, the patients were stratified according to the presence of the high risk HLA-DQ2 heterodimer. The CTLA-4 AA genotype raised to 81.5% in the DQ2 negative group showing a difference statistically significant versus both controls ($p=5.2x10^{-4}$) and DQ2 positive patients ($p=4x10^{-3}$). In the families where the affected child was DQ2 negative, the A allele resulted transmitted in 8 out of 9 informative cases ($\chi^2_{TDT}=5.4$). In conclusion our study confirms CTLA-4 as predisposing gene for CD, with a prominent role in patients without the high risk HLA-DQ2 molecules.

P0035. The potassium-chloride cotransporter 3 gene (KCC3) is excluded from a newly defined 10.9 centiMorgan candidate region for chromosome 15 related schizophrenia (SCZD10)

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Despite the fact that schizophrenia is commonly regarded as a complex disorder, a seperate entry in the Online Mendelian Inheritance in Man Database (OMIM) has been created for chromosome 15 related, hereditary, catatonic schizophrenia (SCZD10, OMIM 605419), which is inherited in an autosomal dominant manner. A possible role of the chromosome 15q14-15 region in the pathogenesis of schizophrenia and manic depressive disorder has recently been reported by several scientific groups. Our group is currently investigating two large families with catatonic

schizophrenia, both families support strongly the chromosome 15 locus. Genotyping of family members did lead to the definement of a 10.9 centiMorgan region between genetic markers D15S1042 and D15S659, respectively. A number of genes expressed predominantly in the brain, and localized adjacent to this chromosomal region, are excluded from the candidate gene panel by mutational analysis and the new fine mapping data. These include genes encoding the nicotinic alpha-7 receptor subunit, the potassium-chloride cotransporter 3, the ryanodine receptor 3, and connexin 36. All these proteins are important factors for brain development and function. Other genes, like the gene encoding the NOTCH ligand DLL4, remain potential candidates. We are currently narrowing down the region of interest by investigating more families and defining the gene(s) responsible for the disease by mutational analysis.

P0036. Association between TNFR2 and familial but not sporadic rheumatoid arthritis provides evidence for genetic heterogeneity.

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Background. Tumor necrosis factor alpha (TNFa), involved in rheumatoid arthritis (RA) binds TNFR1 and TNFR2 receptors. Genome scans have suggested the TNFR2 locus as a candidate RA locus. A case-control study in a UK Caucasian population has shown an association between a TNFR2 genotype (196R/R in exon 6) and familial, but not sporadic RA. Objective. To test this association in the French Caucasian population.
Methods. To test for an association in sporadic RA, 100 families were genotyped for the 196M/R polymorphism and analysed using the transmission disequilibrium test and the haplotype relative risk (HRR). To test for an association in familial RA, RA index cases from affected sib pair (ASP) families (n = 100) were genotyped for 196M/R. Linkage analysis was performed with 3 TNFR2 microsatellite markers.
Results. The TNFR2 196R/R genotype was not associated with sporadic RA (P= 0.72), but with familial RA (P= 0.026). The association was most marked in the context of TNFR2 "twin-like" RA sibs (affected sibs sharing both TNFR2 haplotype) (P= 0.0017). Linkage analysis was consistent with the association, the subgroup of families with 196R/R ASP index cases provided most of the TNFR2 linkage evidence.
Conclusion. This study represents the first replication of the involvement of TNFR2 in RA genetic heterogeneity. Our data refine the initial hypothesis, to suggest that a TNFR2 recessive factor, in linkage disequilibrium with the 196R allele, plays a major role in a subset of families with multiple RA cases.

P0037. Mutations in MKKS gene in two Spanish families with Bardet-Biedl Syndrome

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Bardet-biedl syndrome (BBS) is a genetically and clinical heterogeneous disorder/>Until now six BBS loci have described; BBS1 on 11q13, BBS2 on 16q21, BBS3 on 3p112, BBS4 on 15q22.2-q233, BBS5 on 2q31, and BBS6 on 20p1122 with evidence for at least one more locus. For locus BBS6, the gene has been described and characterized as a chaperonin protein, with a suggested role for protein processing in limb, cardiac, and reproductive system development. Mutations in this gene are responsible for Mc Kusick Kauffman disease and Bardet Biedl syndrome.
Recently a new hypothesis has been proposed, a model of triallelic inheritance, in wich three mutant alleles segregate with the disorder. We found three families out of 20 (15%) with anormal electrophoretic pattern in the SSCP. For family M-176 we found several variations, in exon 3 two polymorphism, silent mutations P39P and I178I, and an insertion of a C in position 938 of the cDNA that leads to a stop codon three codons ahead in position 20 of the protein. In exon 6 we found the two polymorphism described R517C and G532V and an insertion of a G in position 2557 of the coding region that leads to a stop codon 547nt.
For the family M523, we found the same allele of the later family in exon 6 with R517C, G532V and 547stop codon. In exon 4 we found another change in the intronic region of exon 3 a transition of a adenine to guanine .

P0038. Chromosome 22 investigation in rheumatoid arthritis using GenScore, a tool for candidate gene ranking in complex diseases.

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Multifactorial diseases such as rheumatoid arthritis (RA), the most frequent autoimmune disorder, involve several susceptibility genes. Typically, genome scans only provide suggestive loci, resulting in a large number of candidate genes. The aim of this work was to develop GenScore, a new tool for candidate genes ranking in complex disease studies, integrating functional and linkage data. Equal weights were given to both components, producing a GenScore ranging from 0 through 16. RA GenScore was determined for the 247 chromosome 22 genes which function was known, using our 3 cM resolution linkage data (22 microsatellites typed in 88 ASP families). The 5 prioritized genes were IL17R, MIF, IL2RB, CG12-1 cytokine and IGL gene-segment (GenScores ranking from 6 through 7). We propose GenScore as a new tool for candidate genes ranking in multifactorial diseases. Results obtained on chromosome 22 for RA will be posted at www.GenHotel.com.

P0039. Linkage disequilibrium mapping and sequence analysis of a psoriasis locus, PSORS5, on chromosome 3q21.

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Psoriasis is a chronic skin disorder affecting 2% of the population in northern Europe. The disease is characterised by hyperproliferation of keratinocytes and inflammatory infiltration. Psoriasis is today regarded to be a multifactorial disease with a complex genetic background.
In order to identify genetic alterations rendering predisposition to psoriasis several genome scans have been performed. This has led to the identification of several candidate loci but as of today, no single gene have been identified as disease-causing.
The psoriasis susceptibility locus PSORS5 on chromosome 3q21 was identified by our group in a genome-wide screen using a Swedish nuclear family set of 134 affected sib-pairs. Linkage was mainly found in families originating from south-west Sweden and the disease locus is likely to be caused by a founder mutation.
In order to fine-map the PSORS5 locus an SNP map spanning 900-1200 kb was created. A total of 26 SNP markers were genotyped for a large number of Swedish families. The transmission/disequilibrium test (TDT) was used to assess linkage disequilibrium between marker and disease. Five of the 26 SNPs showed significant association ($p > 0.05$). All five markers are located within a 160 kb region.
This region have been screened for disease-involved polymorphisms by direct sequencing of coding regions, association analysis and expression studies of candidate genes.

P0040. Mutational analysis of CARD4/NOD1 gene and Inflammatory Bowel Disease

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Background : IBD are complex genetic disorders of unknown etiology. We have recently identified CARD15/NOD2 as being a susceptibility gene for Crohn's disease (CD).
Aim : Because CARD4/NOD1 shares many structural and functional similarities with CARD15/NOD2, we tested its putative role in Inflammatory Bowel Disease (IBD).
Patients and methods : The IBD families were recruited through a large European consortium. The 11 exons and intron-exon boundaries of CARD4/NOD1 were screened for the presence of variants in 77 unrelated IBD patients (62 CD and 15 UC patients) using direct sequencing. The genotyping of identified variants in IBD families was carried out using a PCR-RFLP procedure and the Transmission Disequilibrium Test (TDT) was computed by GENEHUNTER 2.0 program.
Results : Nine sequence variations were identified in the coding sequence of the CARD4 gene. Five of them (E266K, D372N, R705Q, T787M and T787K) were non conservative variants but only one (E266K) was present in more than one IBD patient. This variant was genotyped in 373 IBD families including 235 CD, 57 Ulcerative Colitis (UC) and 81 mixed families. TDT failed to demonstrate any association between the E266K variant and any of the three phenotypes : IBD, CD and UC ($p>0.05$). The analysis of the phenotype-genotype relationship do not show any specific characteristics neither in patients who are homozygous for E266K, nor in those carrying the 4 other non synonymous variations (D372N, R705Q, T787M and T787K).
Conclusion: These results suggest that CARD4/NOD1 do not play a major role, if any, in IBD genetic susceptibility.

P0041. Interactive effect of DQA1*0101 and the HB*14 AChRα subunit polymorphism on anti-AChR autoantibodies in autoimmune Myasthenia Gravis.

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The HB*14 microsatellite allele located within the CHRNA gene, that encodes the alpha subunit of the muscle acetylcholine receptor (AChR), the HLA classe II DQA1*0101 allele and the DR3 haplotype were previously associated with an increased risk of acquired autoimmune generalized Myasthenia Gravis (MG) using a case-control design. We looked for an influence of these three markers on anti-AChR antibody titers of 480 seropositive MG patients by ANOVA. We took thymus histopathology into account since it markedly influences anti-AChR antibody titers. Their distribution was normalized by logarithmic transformation. A synergistic effect of HB*14 and DQA1*0101 on autoantibody titers was observed ($P<0.03$). In DQA1*0101+ patients, the increase in autoantibody titers associated with the presence of HB*14 was 10-fold in average in the subgroup of thymectomized patients with thymus hyperplasia or with a normal thymus ($P=0.0006$). Consistent with these findings, HB*14 was preferentially transmitted to DQA1*0101+ MG patients of this subgroup ($P=0.031$). This effect of HB*14 was enhanced in haploDR3+ patients, with a 18-fold increase of their autoantibody titers ($P=0.0006$). Moreover, patients with high levels of autoantibody titers (>100nM) had an increased frequency of these three markers (OR=15.71 ; $P=0.0032$). Our data strengthen the hypothesis of an immunological role of the CHRNA gene product in MG predisposition. HB*14 would influence the immunogenicity of the alpha subunit of the AChR, perhaps through presentation of a novel epitopic variant by the DQA1*0101 gene product. This effect would be enhanced in the context of the DR3 haplotype, that would provide non-antigen specific immune dysregulation.

P0042. CARD15 mutations in families with "mixed" Inflammatory Bowel Disease

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Introduction: Recently, the IBD1 locus on chromosome 16 has been identified as CARD15, which encodes an intracellular protein involved in NFkB activation and apoptosis. It has been shown that mutations in the CARD15 gene have been associated with Crohn's disease (CD) but not with Ulcerative Colitis (UC) or in controls.
Aim: To assess the relative importance of CARD15 mutations in « mixed families » where CD and UC co-existed.
Patients and methods: The entire coding sequence of CARD15 gene was screened either by sequencing or by dHPLC in an European cohort of 167 patients (85 CD and 82 UC) from 78 mixed families and 103 healthy controls (HC). Allele frequencies in CD and UC groups were compared with those in HC using the chi-square test.
Results: Twenty-six variants were identified, including the 3 main CD-associated variants (R702W, G908R and 1007fs) in the CD and UC groups. Ten rare mutations identified in both groups were considered as potential disease causing mutations. The allele prevalence of the three main variants R702W, G908R and 1007fs was 4.1%, 4.7% and 8.8% for CD patients; 1.8%, 4.3% and 3.0% for UC patients and 4.4%, 1.0% and 1.9% for controls, respectively.
Conclusion: In the « mixed » families, the G908R variant was associated to both CD and UC ($p=0.03$ and $p=0.04$, respectively), whereas the variant 1007fs was only associated to CD ($p=0.002$). In contrast, the allele frequency of R702W which was found associated to CD did not differ significantly in the CD group and the controls.

P0043. Haplotypic and cladistic analyses revealed a marked dispersion of CD-predisposing nucleotide variations in CARD15/NOD2

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Crohn's disease (CD) is a worldwide early-onset complex condition of uncontrolled inflammation of the gastrointestinal mucosa. We have recently identified three dominating CD's predisposing variants (Arg702Trp, Gly908Arg, Leu1007fs) within *CARD15/NOD2* gene (Nature, 411 : 599-603, 2001). CARD15 is a new member of the CED4/Apaf1 superfamily. The more drastic mutation, Leu1007fs, has been established as a causative variant with a loss of LPS-induced NFkappaB activation. The role in pathogenesis of 32 others non-conservative amino acid changes are currently investigated.
In order to explore the origin and dispersion of CD-predisposing alleles, a total of 232 CD families were recruited through a large European consortium : France (n=128), Belgium (n=23), Scandinavia (Sweden and Denmark, n=17), Mediterranean area (Italy, Spain, Portugal and North of Africa, n=37), Ireland (n=2), Poland (n=2),

originating from France and another country (n=20), and from India (n=1), Sri-Lanka (n=1) and Iran (n=1). All these families were genotyped for 15 markers in and nearby *NOD2/CARD15* including the three non-synonimous sites described above. Haplotypes were built using Haplodump implemented in the GeneHunter package. We report herein a distinct origin of Arg702Trp, Gly908Arg, Leu1007fsinC. The allele frequency of Arg702Trp, and Leu1007insC is partitioned within Europe (p=0,05 and p=0,02 respectively), which is consistent with recent and possibly multiple recurrent mutations events. Susceptibility haplotype distribution in Scandinavian CD families is statistically marked (p<0.05), which could reflect by genetic heterogeneity. In addition, we make optimal use of flanking SNPs to perform cladistic analyses illustrating the importance of phylogenetic analyses and present-day haplotypes complexity for gene-mapping strategy.

P0044. GenHotel, a new approach for candidate gene studies in multifactorial diseases applied to rheumatoid arthritis.

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Introduction : Genome scans in multifactorial diseases result in a large number of candidate genes. To facilitate candidate gene investigation for rheumatoid arthritis (RA), the most common autoimmune disease, we set up GenHotel : the invitation to come and test hypotheses on a common resource (www.GenHotel.com).
Aim of the study : to illustrate the GenHotel approach with the test of RA associated HLA-DRB1 alleles.
Patients and methods: DNA of 100 caucasian French families with one RA patient and both parents were
genotyped for HLA-DRB1. Analysis was performed with the haplotype relative risk (HRR) and the transmission disequilibrium test (TDT). P < 0.05 was considered suggestive and < 10-6 demonstrative.
Results:
HRR : the allele frequency of RA-associated alleles (A) was 57% in transmitted chromosomes versus 28% in non-transmitted, chi 2 = 35,6 (P<10-7)
TDT : out of 97 heterozygous parents A/X, the A allele transmission was 79%, versus 50% under Mendel's lawx, chi 2 = 33,5 (P<10-7)
Conclusion: The results demonstrated the HLA-DRB1 contribution to RA. The GenHotel appoach advantages include genotype quality control, increased robustness from familial based analysis and ability to test complex hypotheses involving haplotypes, inprinting and gene interactions.

P 2. Cancer Genetics

P0045. Germline Mutations in BRCA1 and BRCA2 genes in the Czech Hereditary Forms of Breast / Ovarian Cancer

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Background: It is estimated that 5-10% of all breast cancers can be of hereditary origin. Germline mutations in highly penetrant cancer susceptibility genes BRCA1 and BRCA2 could cause genetic predisposition to breast and ovarian cancers.
Material and Methods: Molecular genetic analysis of BRCA1 and BRCA2 genes was performed in 150 high-risk breast and breast/ovarian cancer families and in 25 women diagnosed with early-onset sporadic breast cancers below 40 years. Protein truncation test and heteroduplex analysis followed by sequencing was carried out on genomic DNA isolated from blood samples. The genetic counseling and preventive clinical follow-up of gene carriers is part of the genetic program.
Results: A germline disease causing mutation was found in 63 screened high-risk unrelated families (42%), 41 mutations (12 different) in BRCA1 gene and 22 mutations (15 different) in BRCA2 gene. The most frequently detected mutations in BRCA1 gene were 5385-5386insC (14 families) and 3819-3823del5 (7 families). Two novel frame shift mutations were detected in BRCA1gene: 2616-2617ins10; 3761-3762del2. Three novel frame shift mutations were detected in BRCA2 gene: 5073-5074del2; 6677-6678del2; 6866delC. Within sporadic early-onset breast cancer cases no disease-causing mutation was found.
Conclusion: Germline mutations in BRCA1 and BRCA2 genes are responsible for a significant fraction of familial breast and ovarian cancer cases in the Czech Republic.
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P0046. Screening of the BRCA1 and BRCA2 genes in western Sweden

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Germline mutations in the hereditary breast/ovarian cancer causative genes BRCA1 and BRCA2 are considered to constitute approximately 6-10% of these cancers. The frequency of female mutation carriers with breast/ovarian cancer depends on the population studied, and display considerable variation in coincidence with ethnic and geographical diversity. Mutations are mainly found as small insertions, deletions or substitutions, but also as exon-wide deletions. We performed mutation analyses in 116 patients, selected under informed consent, from the Sahlgrenska University Hospital, Gothenburg, Sweden. The genes were initially screened using the Protein Truncation Test (PTT) on genomic DNA (BRCA1 exon 11, BRCA2 exon 10, 11) and cDNA from RT-PCR (all other exons) for truncating mutations. All mutations but one was detected with PTT; the remaining one with dHPLC. Automated DNA sequencing of the detected mutations revealed seven different frameshift mutations, two nonsense mutations and one large deletion. Four of these have not been reported earlier: BRCA1 409-410delCA; 2229-2230delAA; 3029delA; 1912 T>G. BRCA mutations were found in 34% of the screened.families; this is comparable to frequencies reported in other European studies. Notably, a western Swedish founder mutation (BRCA1 3171ins5) accounted for 27 of the 37 mutations detected in the 116 families. Our results are furthermore in accordance with the observation that frameshift mutations in the first two-thirds of BRCA1 are associated with a higher risk of ovarian relative to breast cancer than are truncating mutations in the last one-third of the gene.

P0047. An investigation into the methylation status of oestrogen receptor beta and its subsequent expression in human breast cancer

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Since the discovery of the novel oestrogen receptor beta (ERß) much investigation has centered on the role of this new marker in the prognosis of breast cancer and response to adjuvant tamoxifen. The aim of this study is to assess the methylation status of the ERß promoter with respect to ERß expression by immunohistochemistry in a preliminary cohort of 25 primary human breast tumors. In addition we sought to correlate ERα, ERß and c-erbB2 status with tumor staging and prognosis.
Fresh tissue was prospectively collected from screen detected and symptomatic palpable breast tumors managed in the Mater Misericordiae and Mater Private Hospitals. DNA was extracted from the frozen tissue using the standard phenol-chloroform approach. The DNA was subsequently quantified by spectrophotometry and its integrity verified on a 1% agarose gel. To allow Methylation Specific PCR, the DNA was modified by a sodium bisulphate procedure and amplified using methylation specific primers. Resultant products were analysed on an 8% PAGE and confirmed with sequencing.
Table 1. Histological diagnosis and immunohistochemical status

Diagnosis	Total	ERα status (% positive)	ERß status (% positive)
Invasive Ductal	22	20/22 (91%)	16/22(73%)
Invasive Lobular	3	3/3 (100%)	2/3(66%)

With the establishment of this novel MSP approach to analyzing the methylation status of the ERß promoter in human breast cancer, we intend to correlate the findings with the immunohistochemical results obtained with the 14C8 monoclonal antibody. These studies are of special importance in the context of epigenetic reversibility as a potential therapeutic option.

P0048. Analysis of mutations in the BRCA2 gene in Chilean families with breast cancer

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Two genes have been described until today as responsible for familiar breast cancer, BRCA1 and BRCA2. Several studies have demonstrated that the frequency of mutations in either gene is variable depending on the population analysed. Since this effect may be due to the ethnic origin of the families selected, we were interested in knowing the incidence of BRCA1 and BRCA2 mutations in Chilean families. We previously reported a very low frequency of mutations in the BRCA1 gene (10%). This study shows the analysis of BRCA2 mutations in the same group of families. The families were selected by standard criteria. All exons encoding the BRCA2 gene were PCR amplified and analysed through SSCA, heteroduplex and DNA sequencing. We found in one family the 6174delT mutation in exon 11, which has been extensively reported in families with Ashkenazi-Jewish ancestors. The patient carrying the mutation informed about Ashkenazi-Jewish ancestors in her family. The second mutation found is 6857delAA, also present in exon 11. This is a very interesting mutation for our study, since it has been described previously in three families from Spanish origin. Due to high percentage of admixture of the Chilean population with Spanish colonisers during the XVI and XVII centuries, it is highly probable that the mutation has a common ancestor. Also it is interesting to note that the incidence of BRCA1 and BRCA2 mutations in the Spanish population is below 20% each. (Financed by FONDECYT 1011076)

P0049. Telomerase enzyme activity and chromosome abnormalities detected by combined G-banding and comparative genomic hybridization in primary breast cancer

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Several structural and numerical chromosomal abnormalities have been recorded in breast cancer. It has been suggested that some chromosomal aberrations may be the result of telomere dysfunction in rapidly proliferating cells. The de novo activation of telomerase in cancer possibly provides a survival mechanism curtailing further chromosomal aberrations. In order to investigate the relation of telomerase level of expression and the extent of chromosomal aberrations, 62 primary breast carcinomas were studied. Telomerase activity was measured using a PCR based TRAP assay and 92% of the tumors were found to express Telomerase with relative activity ranging from 0 – 3839.6. Genetic alterations were determined by G banding and Comparative Genomic Hybridization analysis. 97% of the tumors exhibited chromosomal aberrations ranging from 0-45. The average number of genetic alteration recorded by G-banding and CGH was 10.98. No correlation was observed between Telomerase activity levels and the number of genetic alteration in the overall sample population. However when tumor samples with below average genetic alteration numbers were considered as a separate group, a statistically significant positive correlation was recorded between Telomerase activity levels and number of genetic alterations (R=0.339, p=0.032). This relationship was inversed in the sample group with above average genetic alterations but it was not statistically significant (R=-0.127; p=0.574). These results suggest that Telomerase may be activated in the initial steps of carcinogenesis perhaps to maintain chromosomal integrity of the rapidly proliferating tumor cells while at the later stages alternative survival mechanisms have evolved.

P0050. Selective genetic screening in 250 Belgian breast and ovarian cancer families identifies BRCA1 or BRCA2 mutations in 20% of cases

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Germline mutations of the BRCA genes are associated with familial breast and/or ovarium cancer, or with early onset of breast cancer (<35y) in the absence of a familial history. In families attending a cancer genetic clinic mutations are typically identified in 15-20% of the analysed families. Most mutations are truncating and spread all over the coding regions, but at a higher frequency in the large exons (exon 11 of BRCA1 and exons 10 and 11 of BRCA2). We chose to analyse these large exons together with exons with Belgian founder mutations, Ashkenazi mutations, hot spots and exonic deletions. DNA samples of a cohort of 250 unrelated affected patients were included in this study: 140 samples were analysed by Enzymatic Mutation Detection (EMD), while 110 samples were screened by Denaturing High Performance Liquid Chromatography (DHPLC). Exon deletions were analysed by amplification across breakpoint junctions.
By screening selected exons of BRCA1 and BRCA2, 21 germline truncating mutations and one exonic deletion were found in 48 of 250 families. In addition, one pathogenic missense mutation has been identified in BRCA1: M1V. DHPLC has advantages in comparison to EMD, mainly because it is semi-automatable. This study shows that a limited screening of BRCA1 and BRCA2 results is a high yield of mutations in our clinical sample (20%). This screening could be offered to a large group of females while an exhaustive screening could be performed in a more selected group. Analysis of additional exons by DHPLC is going on in such a selected group.

P0051. RAD6 and breast cancer

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Treatment with DNA damaging drugs is commonly used to localize breast cancer. It causes DNA damage and leads to genomic instability and/or apoptosis as a result of mutation or altered expression of genes associated with DNA repair, in particular RAD6. RAD6 (Ubch2) protein is present at low amounts in cytoplasm of normal human breast cells, while in metastatic breast cancer cells it is up-regulated and localized in the nucleus. We have demonstrated, that overexpression of exogenous RAD6 cDNA in MCF10A human breast epithelial cells induced cell-cell fusion generating multinucleated cells, centrosome amplification, abnormal mitosis and aneuploidy. We found that exposure of MCF10A cells with cisplatin or adriamycin resulted in enhancement of RAD6 mRNA/protein level which was post-transcriptionally regulated and post-translationally stabilized. RAD6 protein is predominantly expressed during late S/G2 phases. Its localization in cells at specific stages of mitosis reveals the lack of association of RAD6 with condensed chromatin. Co-localization of RAD6 protein with γ-tubulin on centrosomes is maintained throughout the interphase and mitotic stages of the cell cycle. We were able to show for the first time that in drug-treated cells RAD6 is associated with p53-p14ARF-MDM2 in the nucleus. The expression of RAD6 correlates with human breast cancer stage and can be used as a marker to predict response to chemotherapy. Our findings suggest that RAD6 at low levels may play a significant role in the maintenance of genomic integrity of mammalian cells, high levels of RAD6 probably over a certain threshold may cause genomic instability.

P0052. Elevated CA-125 serum level as an example of correlation between cell biology, carcinogenesis, positive family story. A case of woman from the breast/ovarian cancer family, with mutation in BRCA1 gene.

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Characteristic feature of the neoplasm evolution is long-lasting process. The priority of oncology is to diagnose cancer in its pre-clinical stage of development. It is extremely important in families, which have positive family story. The point is searching for substances, presence of which in the blood would give evidence to the presence of cancer. In case of CA-125, its production in tumor is significantly higher than in a normal cell. Level depends on mass of

the living tumor cells.
Here we report a case of patient, whose family was affected by three breast and one ovarian cancer. Because strong aggregation breast and ovarian cancer- the woman performed genetic test. It showed mutation in BRCA1 gene, exon 20-5382insC. The next two mutations were proven in her sister daughters.
In all performed clinical examinations (mammography, ultrasonographic examination of the breasts, transvaginal ultrasonography) there were no pathology. But the serum level CA-125 was highly increased, accordingly:108 and 125 IU/L (normal: 30 IU/L). She underwent prophylactic oophrectomy, and at the time of surgery tumor was suspected. Histopathology gave the final solution: poorely diffrentiated adenocarcinoma, only in one site of the left ovary. As a consequence, the woman started few courses of chemotheraphy.
We want to conclude, that we should perform BRCA1 and BRCA2 tests in families with strong aggregation of breast and ovarian cancer. We should have a high suspicion of cancer, when serum level of Ca-125 is highly increased. We want to undreline the strategy of prevention.

P0053. Androgen Receptor CAG Repeat Length in Jewish Israeli Women who are BRCA1/2 Mutation Carriers: Association with Breast/Ovarian Cancer Phenotype

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BRCA1/2 mutation carriers are at increased lifetime risk for developing breast and/or ovarian cancer. Yet, the genetic or environmental factors that govern the phenotypic expression of mutant BRCA1/2 alleles remain elusive. The CAG repeat, within exon 1 of the Androgen Receptor (AR) gene is reportedly associated with breast cancer phenotype in BRCA1 mutation carriers. To extend this observation, we genotyped 227 BRCA1/2 mutation carriers for the polymorphic AR CAG repeat, and correlated allele size with breast/ovarian cancer morbidity parameters. Of 227 BRCA1/2 carriers, 169 were BRCA1 mutation carriers and 58 carried a BRCA2 mutation. Seventy-nine women had unilateral breast cancer, 15 - bilateral breast cancer, 41 - ovarian cancer, 14 - breast and ovarian cancer and 78 were asymptomatic mutation carriers. Mean age at diagnosis in women with either or both neoplasms was 46.7±11.2 years, and that of the asymptomatic group - 45.8±9.4 years, a statistically insignificant difference. The AR CAG repeat ranged from 8-28 in all tested women. Mean number of AR CAG repeat was not statistically different between affected (18.3±2.4) and asymptomatic mutation carriers (18.6±2.1). AR CAG repeat among patients with early onset (<42 years) breast cancer was significantly shorter (17.5±2.3) compared with asymptomatic individuals (18.6±2.1) ($p<0.01$), and the shorter allele - the younger the age at diagnosis. This study does not provide conclusive evidence of association between AR CAG repeat size and breast or ovarian cancer risk. However, a small effect of a short AR CAG allele size on breast cancer penetrance at early age was noted.

P0054. Association of 5382insC Mutation with SNPs of BRCA1 Gene and the Mutation Frequency in Russia

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A high predominance of 5382insC in BRCA1 gene mutation spectrum (80% of all mutations) of patients with familial breast/ovarian cancer and a set of 11 SNPs on an extent of the gene were found. This set of SNPs in strong linkage disequilibrium and consensus sequence defined two most frequent haplotypes (named B and A). The haplotype frequencies in cancer patients and in control individuals were not different significantly. However, the haplotype A to the haplotype B ratio in genomes with the 5382insC mutation was approximately three times higher than these haplotypes ratio in the population ($P < 0.04$). The same difference between patients under 5382insC mutation and control group was observed for genotype frequencies ($P < 0.04$) with odds ratio equal 3.3 in favor of AA among patients. The reason for observed frequency difference may be the mutation - haplotype A linkage. But it is interesting that a ratio of genotype AA and AB frequencies under the mutation is different in patients and control individuals. This may suggest on operation of other factors in addition to linkage. It should be noted that the haplotypes A and B frequencies in Russian and West-European patients with 5382insC were the same ($P = 0.40$).
The frequency of 5382insC mutation revealed in Russia is highest among investigated populations. The proportion of this mutation is next highest in East-European countries and common in West-Europe. These data jointly with the same SNP haplotypes found in Russia and West-Europe are suggestive on East-European origin of 5382insC mutation.

P0055. BRCA2 mutations and polymorphisms in Russian patients with familial breast/ovarian cancer

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BRCA1 mutations were found in 40% of a sample of breast/ovarian cancer families in Russia. In present study BRCA2 gene sequence variations among cancer families of the rest part of the sample were investigated.
There were only 11% of the probands with deleterious BRCA2 mutations. Two deleterious mutations - 2001del4 and 4816insG - are new. Missence mutations of unclear significance were revealed in 19% of the cases. One of that (N1808K) and two single nucleotide polymorphisms (SNPs) are revealed for the first time. The gene variant S384F that was thought to be unclear significance mutation is evidently polymorphism because was found in common with deleterious mutation of BRCA2 gene. SNPs of 12 types on an extent of BRCA2 gene were found. Six of those were in coding regions of the gene. A variant N372H that known as confers an increased breast cancer risk under HH homozygote, in 12% of the cases was homozygosis on HH with allele frequency equals 0.31. At the same time, a variant IVS11+80del4 with the similar allele frequency was not found as homozygote. It is interesting that we found a frequency of 203G/A polymorphism significantly higher in comparison with results in BIC data base, although the frequencies of other frequent SNPs were not so different. BRCA2 SNPs of control group are under investigation at present.

P0056. Prevalence of BRCA1 gene 5382insC mutation in St.Petersburg patients with familial breast cancer.

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The BRCA1 gene mutation are the common cause of familial breast cancer. The risk of breast cancer development in women with germline BRCA1 gene mutations approaches 90% during life span. We have created the DNA collection from St. Petersburg familial breast cancer patients and demonstrated nearly the same frequency of BRCA1 gene 5382insC mutation in both Slavic and Ashkenazi Jewish patients with familial breast cancer. 5382insC mutation of the BRCA1 gene was found in 1 Ashkenazi Jewish family with familial breast cancer out of 9 studied and in 3 Slavic families with familial breast cancer out of 20 studied. 5382insC mutation was found neither in Ashkenazi and Slavic patients with sporadic breast cancer, nor in control group, that consists of 50 Slavic and 50 healthy Ashkenazi patients unselected in respect of familial breast cancer. Previously 5382insC mutation of the BRCA1 gene was reported in number of familial ovary cancer patients from Moscow and thus 5382insC mutation of the BRCA1 gene may be the common cause of familial breast and ovary cancer in whole Russia. However, the mutation spectra specificity from other countries in BRCA1 gene is expected in Russian population and some new BRCA1 gene mutations are in process of characterization now. The elucidation of BRCA1 gene mutation spectra in St. Petersburg familial breast cancer will help to provide genetic counseling in breast cancer families and improve

treatment patients with high risk of breast cancer in the future. The current research was supported in part by RFBR grant 01-04-49627.

P0057. Altered expression of the candidate tumor suppressor gene, WWOX, in human breast tumors

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The presence of putative tumor-suppressor genes on chromosome 16q23.2-24.1 has been suggested by LOH analysis in breast cancer as well as other cancer types. This region overlaps with the fragile site FRA16D and the region of homozygous deletions found in various cancers. We have previously constructed a 1.2 Mb contig map and used this resource to assign transcripts to the LOH region. This resulted in the identification of the WWOX/FOR gene.
The mouse homologue of the WWOX protein has been defined as an apoptogenic protein and an essential partner of p53 in cell death. Thus WWOX is a strong candidate tumor-suppressor gene. We have performed an expression study of the WWOX gene in a series of human breast tumors and breast cancer cell lines, and detected altered WWOX expression at high frequency in cancer cells. Furthermore, identification of two distinct alternative WWOX transcripts expressed at high levels in human tumors suggests an involvement of the WWOX gene in cancer progression. We have initiated functional studies of WWOX in human cells in order to characterize the role of the WWOX protein in normal as well as cancerous cells.
This work was supported by the Research Council of Norway, the Norwegian Cancer Society, the Ligue Nationale de Lutte Contre le Cancer (LNCC), and the Association pour la Recherche sur le Cancer (ARC).

P0058. Investigation of APC mutations of a patient with FAP and her family members by heterodublex analyses

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Familial adenomatous polyposis coli (FAP) is an autosomal dominant disease characterised by the presence of 100 or more colorectal adenomatous polyps. Mutations in the adenomatous polyposis gene (APC) gene primarily responsible for the development of this disease. In this study, we examined one patient with FAP and 21 family members including one effected person from FAP and 20 nonsemptomatic persons. Our proband case who have a retinal lesions (congenital hypertrophy of the retinal pigment epithelium, called CHRPE) and hundreds adenomatous polyps on all colon and rectum is a 36 years old woman. We isolated DNA from pheripheral blood samples of proband and her family members by proteinaz K incubation and phenol-chloroform extraction. We studied E,D, F, and G segments of exon 15 of APC gene by heterodublex analyses (HDA). For staining, we used non-radioactive silver staining method. We determined mutation in 5 person from this family in segment F of exon 15 of APC. Two of them were patients with FAP (one is ourproband case) and another three persons were non semptomatic family members. Result of sequencing analysis of these cases, we determined T deletion at position 3554 causing a frameshift mutation in APC gene.

P0059. Involvement of APC/beta-catenin signalling and E-cadherin in sporadic colon cancer

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Activation of APC/beta-catenin signalling pathway by mutation in the APC or beta-catenin gene contributes to colorectal carcinogenesis. E-cadherin is involved in control of intercellular adhesion and acts as an invasion supressor. We examined 60 cases of human sporadic colon cancer and corresponding normal tissue samples to evaluate the loss of heterozygosity (LOH) and presence of mutations at the APC, beta-catenin and E-cadherin gene loci.
DNAs were used for PCR, RFLP, VNTR and LOH analysis. To analyze LOH at the APC gene loci we used three RFLP intragenic markers (exon 11 RsaI, exon 15 MspI, and exon 15 AspHI). The presence of the mutations in the amplicon 15H of the APC gene, and APC gene mutation in codon 1309 were analyzed as well. To analyze mutations in the beta-catenin gene we amplified exon 3 and the intronic sequences flanking it from tumor DNAs. For the LOH analysis of E-cadherin gene locus we used D16S752 polymorphic marker. The informativity for all three APC intragenic markers was 53.3 % (32 of 60 assayed), and 25 % of tumors (8 of 32 informative) demonstrated LOH. We found two APC gene mutations in our tumor samples: a deletion in codon 1309, and an insertion in the amplicon 15H of the APC gene. In 3.3 % of tumor samples (2 of 60 tested) the mutation of the beta-catenin gene was found. The informativity of D16S752 E-cadherin gene polymorphic marker was 75% (45 of 60 tested) and 28.8 % of tumors (13 of 45 informative) demonstrated LOH.

P0060. Genetic analysis of APC gene and the diagnostics of familial adenomatous polyposis in pediatrics

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Familial adenomatous polyposis (FAP) is an autosomal dominant inherited disease. Patients with FAP develop hundreds to thousands of adenomatous polyps in the colon and rectum during their second or third decades and one or more of them can progress to cancer. Children of affected individuals are at 50% risk of inheriting the disease. Because FAP patients have a very high risk of colorectal cancer, identification of the individual risk in family members is important to prevent cancer deaths. For these at risk members of the family, annual endoscopy is recommended. The method of providing such accurate presymptomatic diagnosis is to determine whether a family member has inherited the particular germ-line mutation of the adenomatous polyposis coli (APC) gene carried by the affected parent.
Genomic DNAs were isolated from peripheral blood of patients and their relatives. Polymerase chain reaction (PCR) was performed using specific pairs of primers. PCR products were analyzed by electrophoresis on a Spreadex EL 300 gels.
The genetic analysis confirmed the APC gene codon 1309 germ-line mutation in two children not yet having colorectal adenomas, but having inherited APC gene mutation from their mother who died from colon carcinoma. APC gene mutation analysis also confirmed the diagnosis of FAP in one child having colorectal adenomas as a first case of FAP in that family.
We use APC gene mutations analysis in presymptomatic diagnostics but also to confirm the diagnosis of FAP. Children confirmed as a gene mutation carriers can be early included in surveillance program and treatment.

P0061. Germline mutations of the APC gene in Czech FAP families

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Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited disease characterised by the development of hundreds to thousands of colorectal adenomatous polyps and by the progression to carcinomas. Causative germline mutations have been described in the adenomatous polyposis coli (APC) gene. In the present study the entire APC coding region has been screened for germline mutations in 45 unrelated Czech FAP families. Using PCR, DGGE analysis and DNA sequencing we found 30 mutations, twelve of which were found to be novel: seven frameshift mutations (exon 9 and 15), two nonsense mutations (exon 9 and 11) and three splicing mutations (intron 11 and 14). In previously reported mutations we identified ten frameshift mutations in exon 15 and six nonsense mutations (exon 5, 9 and 15). The common 5 bp deletions at codons 1309 and 1061 were identified in five families (16,6%) and substitution 2805C>A was found in three cases (10%). Identified mutations result in the classical form of FAP except the 1 bp deletion at codon 409 (exon 9),

which caused the attenuated form of FAP. In addition, one missense mutation was revealed in exon 3 although missense mutations are rarely observed in FAP. It remains to be determined whether this substitution represents the disease-causing mutation. Two samesense variations were detected in exon 15. The presence of one of them correlates with disease occurrence in the affected family. Thus the clinical significance of this mutation cannot be excluded.
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P0062. Denaturing High-Performance Liquid Chromatographie (DHPLC) in Screening for Mutations in Exon 15 of the APC (Adenomatous Polyposis Coli) Gene

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FAP (OMIM: *175100, McKusick 1986) is a rare form of hereditary colorectal cancer. Germline mutations of the APC gene were reported in patients with Familial Adenomatous Polyposis (FAP). Inactivation of the APC gene plays a significant role in the development of early onset colon cancer based on polyposis of the colorectum. The location of germline mutations in the APC gene appears to correlate with the clinical phenotype (number of colorectal adenomas, concomitants like occurence of further adenomas in other digestive organs, desmoid tumors and Congenital Hypertrophy of the Retinal Pigmental Epithel [CHRPE]). To provide a fast mutation screening we analyzed the region of the APC gene where more than 40% of the mutations in FAP are described (exon 15-4 to 15-8). We established DHPLC (Denaturing High Performance Liquid Chromatography) mutation analysis followed by automated sequencing of suspicious fragments. We investigated 9 patients with a clinical diagnosis of FAP. Three sequence variations could be identified: 1 polymorphism and 2 mutations (3597del2A at codon 1199 with termination of protein translation at amino acid position 1206; 3949GàC at codon 1317, E1317Q). We describe the optimized conditions for DHPLC for this gene. According to our results DHPLC is an efficient and fast screening method to identify mutations in the APC gene which can be applied to the other exons of the APC gene for a fast and cost reducing mutation screening. The rapid mutation screening will optimize further diagnostic and therapeutic strategies in families with hereditary colon cancer.

P0063. NF1 tumor suppressor gene in sporadic colon cancer

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Colorectal carcinomas are characterized by multiple genetic alterations that occur during tumorigenesis. Several tumor suppressor genes associated with colorectal carcinoma have been identified: MCC and APC on chromosome 5q, p53 on chromosome 17p, nm23-H1 on chromosome 17q, and DCC and DPC4 on chromosome 18q. We examined 60 cases of human sporadic colon cancer and corresponding normal tissue samples to evaluate the loss of heterozygosity (LOH) at the NF1 gene loci. The purpose of this study was also to evaluate whether the LOH at the NF1 gene is associated with clinicopathological characteristics in sporadic colon cancer. DNAs were used for PCR, RFLP, VNTR, and LOH analysis. PCR was performed using specific pairs of primers. PCR products were analyzed by RFLP analysis, and VNTR analysis. To analyze LOH at the NF1 gene loci we used three polymorphic markers: one RFLP marker (exon 5 RsaI) and two VNTR markers (IVS27AAAT2.1 and IVS38GT53.0).
Using these three polymorphic markers 50 (83.3%) patients were found heterozygous and informative for LOH analysis. DNA from 9 (18%) tumors exhibited LOH at the NF1 locus. The majority NF1 gene LOH was observed in Dukes' A (56%), in the well differentiated tumors (43%), and in the tumors that were smaller than 5cm (67%). Conclusion: Our results support the view that malignant progression is a consequence of more than one genetic change and suggest that inactivation of NF1 gene plays a role in a multistep process of colon tumor progression as an early event.

P0064. Complete characterization of the colon cancer cell line HT29 clone 19A by multicolor banding (MCB)

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The human colorectal adenocarcinoma cell line HT29 subclone 19A was recently characterized by M-FISH (Eur J Hum Genet 2001, Vol 9/S1, p138, P0193) and the following composite karyotype was established:
64~69,XX,+del(Xp),+1,+der(1)t(1;11;16),+2,+der(2)t(1;2),+der(3)ins(3;12),+der(4)t(2;4),+5,+del(5q),+7,+7,-8,+dup(8),+del(9),+der(9)t(6;9;X;9),+10,+11,+11,+del(11p),+del(11q),+12,-13,-13,+i(13q),+i(13q),+der(13)t(5;i(13q)),+15,+16,+17,+del(18),-19,+del(19),+der(19)t(5;19),+der(19)t(17;19),+20,+20,+22,+22[cp10]. Using M-FISH, it was possible to identify the chromosomes involved in aberrations, but not to define their exact breakpoints. In order to further clarify the translocation breakpoints and to characterize possible iso-chromosomes, multicolor banding (MCB) was applied at the 400 band level according to Mrasek et al. (Cytogenet Cell Genet 93:242-248). MCB-analyses were performed on all aberrant chromosomes of this composite karyotype, i.e. on the following eighteen chromosomes: #1, #2, #3, #4, #5, #6, #8, #9, #11, #12, #13, #16, #17, #18, #19, #20, #22 and X. Where necessary for exact definition of rearrangements, MCB-probes were combined with centromere specific and whole chromosome painting probes. The resulting karyotype is as follows:
64~69,XX,+del(X)(p11.2 qter),+del(1)(p35--qter),+2,+der(2)t(1;2)(1q32→1qter;2pter→2q11),-3,+i(3)(q10),+der(3)ins(3;12)(3pter→3p12::12p12::3p12→3qter),+der(4)t(2;4)(2q35→2qter;4pter→4q11),+5,+del(5)(pter→q11.2),+dic(6;9)t(6;9;X;9)(6pter→6q10;9q10→9q21;Xp21.1→Xp11.3;9q21→9qter),+7,+7,-8,+dup(8)(qter→q10::q10→q24::hsr::q24→qter),+10,+11,+del(11)(p13→qter),+der(11)t(11;?)(11pter→11q24;?),+12,-13,-13,+i(13q),+i(13q),+der(13)t(5;13)(13qter→13q10::13q10→13q21::5q31→5qter),+15,+del(16)(pter→q13),+der(17)t(19;17)(19pter→19p11;17p11→17qter),+i(18p),-19,+der(19)t(5;19)(5pter→5p11;19q10→19qter),+20,+20,+22,+der(22)t(17;22;17) [cp10].
In summary, the MCB-technique was suitable to define all translocation breakpoints apart from one (i.e. der(22)t(17;22;17) which consists of only very little chromosomal material). Thus, MCB is a very useful tool for detailed analyses of chromosomal rearrangements.
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P0065. Optimization of DHPLC experimental conditions for mutation analysis of the hereditary non polyposis colon cancer (HNPCC) genes hMLH1 and hMSH2

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Denaturating high-performance liquid chromatography (DHPLC) is an efficient method for the detection of point mutations in disease-related genes.
Colorectal cancer is one of the most common cancers, and mutations in the genes for hereditary non polyposis colon cancer (HNPCC), hMLH1 and hMSH2 represent the major cause of hNPCC.
We have recently applied the DHPLC mutation detection to the 16 exons of hMSH2 and 19 exons of hMLH1 genes. To test sensitivity and reproducibility of DHPLC, we have first determined the best DHPLC conditions on the wild type sequences, followed by the study of 35 sequence variants previously found by sequencing DNA samples of HNPCC patients. All of the 35 mutations were detected using DHPLC (sensitivity 100%).
We then used DHPLC to analyse 18 patients with colorectal cancer not fulfilling all the Amsterdam criteria. We have found two mutations : Y43C in exon 1 of hMSH2 (unpublished yet) affecting a highly conserved residue and a 790+1G to A in intron 9 of hMLH1 (previously described), one of them did not fulfill the Amsterdam criteria. This low mutation yield could be due to the patients inclusion

criteria. We have also found many polymorphisms, with a much higher frequency than previously published.
In conclusion, DHPLC is a rather rapid and inexpensive technology that may be used to screen for mutations colorectal cancer patients where HNPCC may be suspected but who do not fulfill stricter criteria.

P0066. Unusual Findings Of APC Gene Analyses In suspected FAP Cases From The Republic Of Macedonia

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AIM: Characterization of the molecular basis of FAP in Macedonia.
SUBJECTS: Patients with multiple adenomatous polyposis of the large intestine confirmed by histopathological evaluation.
METHODS: DGGE of exons 1-14, PTT and heteroduplex analysis of exon 15, RT-PCR of exons 1-15, sequencing of the 5' end, and Southern blot analysis of the APC gene.
RESULTS: Six unrelated cases (one female and five males) with multiple polyposis of the colon were enrolled in this study. Of the six patients, only one had a positive familial history. In the female patient the number of polyps was much lower (<100) than the number observed in the male subjects (>1,000). Detailed DNA analyses of the APC gene revealed the presence of rare (unusual) defects in two patients. A large deletion, removing the entire APC gene, was found in the patient with a positive familial history. A somatic mosaicism for a deletion removing exons 2-14 was detected in the female patient. No abnormalities at the DNA level and no allelic imbalance in the expression profile of the APC gene were detected in the other four patients. Abnormal APC gene transcripts were found in the peripheral blood of two of these (deletion of exons 9-13 and exon 14, respectively) that could not be explained by any defect at the DNA level.
CONCLUSION: The unusual findings of our study indicate that there are genes other than the APC which might influence the development of multiple colorectal adenomas, either through an APC related mechanism or through other pathways.

P0067. Linkage mapping of FAP disease modifier locus in a large family with a known *APC* mutation

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Familial adenomatous polyposis (FAP) is an autosomal dominant colorectal cancer predisposition syndrome caused by germline mutations within the adenomatous polyposis coli (*APC*) gene. Mouse model studies and broad phenotypic variability observed both within and among affected families indicate, that in FAP disease expression also other genetic and environmental factors must play an important role. Their identification would substantially improve possibilities of genetic counseling of FAP patients by enabling the precise prediction of the disease severity. A large FAP kindred which has been previously reported by our group and harbours an adenine deletion at codon 1982 of the *APC* gene represents an ideal model for studying FAP modifiers. Though carrying the same mutation, the affected subjects (45) present with variable colonic and extracolonic manifestations which are in several branches transmitted through the generations. Performed simulation studies revealed a high potential of this pedigree to detect a modifier locus. Here, the results of linkage analysis of 20 candidate regions and eventually results on the genome wide screening for a modifier gene in FAP condition will be presented.

P0068. Monozygotic twins showing variable expression of Muir-Torre syndrome due to MSH2 mutation.

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Muir-Torre syndrome (MTS) is an autosomal dominant genodermatosis characterized by skin tumors associated with visceral malignancies. MTS shares many clinical similarities with Hereditary Nonpolyposis Colorectal Cancer (HNPCC), and germ-line mutations in DNA mismatch repair (MMR) genes have also been found in MTS families. We present monozygotic sisters with MTS caused by a point mutation (IVS5+3A>T) in the 3' splice site of exon 5 of MSH2 resulting in the deletion of this exon from the mRNA, thus encoding a truncated protein. One sister developed her first squamos cell carcinoma at 50 years, and at age 53 had two sebaceous carcinomas and one adenoma removed. The second sister had uterine cancer at age 40, metastatic colon cancer at 43, thyroid cancer at 45, and sebaceous adenoma at 49 years. Their mother is deceased at the age of 41 from uterine and liver cancer, and the maternal grandfather was diagnosed with colon cancer at 50 years. Kindreds with this same MMR mutation are described in the literature, and the types of cancers represented in these families were quite varied and included colon, uterine, rectal, endometrial, brain, ovarian, ureter, gastric, breast, duodenal, sebaceous, bone, thyroid, and lung cancer. Therefore, genetic counseling must emphasize the inability to establish any correlation between the site of the individual mutation and spectrum of tumor types and the use of appropriate surveillance methods. This family demonstrates the intrafamilial variability of carcinogenesis even among monozygotic twins, and suggests that non-genetic factors are important in modifying the expression of this syndrome.

P0069. Mutations of N- and K-Ras, p53 and FMS genes in myelodysplastic syndromes in children

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Myelodysplastic syndromes arise from molecular-genetic disorder of myeloid stem cell, characterized by dysfunction of myeloid, monocytic, erythroid and megakaryocytic lineages and high risk of evolution to ALL. Hence, MDS are considered to be preleukemic states and are a model for studying mechanisms of leukemic transformation. Ras, FMS and p53 were found to be the most frequently mutated genes in adults with MDS. We have used PCR-SSCP method and sequencing to examine mutations in these genes in 35 archival bone marrow samples of children with MDS collected in last ten years in Institute for Mother and Child Health. 22 DNA samples were successfully amplified with primers for N-Ras (exons 1 and 2), K-Ras (exons 1 and 2) and FMS (region including codon 969) genes and 11 with primers for p53 gene (exons 5, 6, 7, 8 and 9). One of analyzed samples harbored mutation in first exon, and two in second exon of N-Ras gene. In two samples were detected mutations in second exon of K-Ras gene. We have not detected mutations in analyzed regions of neither p53 nor FMS genes. These findings may suggest that mutations of Ras genes play important role in the development of MDS in children.

P0070. The relationship of chromosomes 7,9,10,17 aneuploidies and p53 gene alterations between the low and high grade astrocytomas, using interphase FISH technique

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In the present study, we examined chromosomes 7, 9, 10 and 17 aneuploidies and p53 gene alteration on surgical fresh tissue samples of 29 different grades of astrocytomas by using flourescence in situ hybridization (FISH). Eleven of these astrocytomas were low grade (5 pilocytic and 6 grade II) and eighteen astrocytomas were high grade (6 anaplastic and 12 gioblastoma multiforme). All samples were classified according to the WHO classification of tumours of the central nervous system and none of the patients received preoperative chemotherapy or radiotherapy. The results showed that 2 of 11 low-grade and in 6 of 18 high-grade tumours had trisomy 7. One of 11 low-grade and one of 18 high-grades had monosomy 9. Three of 11 low-grade and in 5 of 18 high-grade tumours had monosomy 10. One of 11 low-grade and 2 of 18 high-grade tumours

had monosomy 17. Three of 11 low-grade and 6 of 18 high-grade astrocytomas had deletion of p53 gene, although there were not monosomy 17. Based on these findings, chromosomes 9 and 17 aneuploidies are not exclusive to low and high grade astrocytomas. However we identified monosomy 10 and p53 deletions similary rates between low and high grade astrocytomas, gain of chromosome 7 was identified in high-grade astrocytomas nearly two times more than low grade ones. Thus, we suggest that loss of chromosome 10 and p53 gene abnormalities to be earlier event than gain of chromosome 7 for carcinogenesis of astrocytomas.

P0071. p53 mutations and PAX5 and SHB genes expression in superficial bladder cancer

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Transitional cell carcinoma belongs to a very heterogenous group of neoplasms. Prognosis of a patient at the time of diagnosis is a basic problem of the bladder cancer therapy and has encouraged the search for prognostic markers. The role and possible oncogenic activity of the PAX genes have been discussed recently. A candidate gene PAX5 is situated on the 9p21-23, the region of the most frequent genetic changes in bladder cancer, as well as another signal transduction gene SHB. Concerning the prognostic potential of p53 mutations, recent research yielded contradictory results. The aim of the study was to define new combinations of prognostic markers reflecting biological behaviour of individual tumours in order to identify patients at risk for tumour progression. We investigated 44 patients with superficial bladder cancer and 20 controls for p53 mutations in exons 5-9 and adjacent intronic sequences by the SSCP and direct genomic sequencing. One mutation (del 128 Pro) was detected among the 44 tumours (2.3 %). 36 patients overexpressed at least one of the PAX5 or SHB genes, 26 of them overexpressed both genes. The staging and grading of these pacients were generally higher then of those without increased expression. The correlation of PAX5 and SHB expression and clinical and histopathological data was evaluated. In conclusion, our results indicate that combination of expression data might be used as a diagnostic tool in superficial bladder cancer. Supported by the grant IGA MZ NC/5961-3.

P0072. Loss of heterozygosity of p53 gene in gastic carcinoma in the region of easten Turkey

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Loss of heterozygosity effecting various chromosomes has been characterized on tumor of many human cancers. Tumor suppressor gene p53 was found to be primer target for that losses. In our study, we examined 41 patients with gastric neoplasm for loss of heterozygozity effecting the p53 gene by using PCR/RFLP technique. The samples were run on to agarose gel and visualized on UV. Cancerous lesion of wet tissues from 25 of 41 patients was taken together with their peripheral blood samples. 6 patients was inoperable, so only blood was taken and paraffin tissue of 10 patients were examined for allelic losses. The PCR was carried out by using two sets of primers, both amplified 72. codon of exon 4 of p53 gene. The primer called G-H gave amplified fragment of 66 bp and the other I-J gave 247bp fragment. These fragment were subjected to restriction enzyme BstUI for detecting LOH. 18 out of 41 patients exhibited heterozygosity loss 43,6 % and many of these LOH positive cases had lenf metastasis. We could not determined any relation between p53 LOH positivity and sex or age. Finally, it has been shown that LOH in p53 gene are common in gastric cancer and play important role for cancer progression.

P0073. Loss of heterozygosity in tumours of carriers of germline TP53 mutations

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A strong genetic determination is observed in about 5% of all cancer cases. These patients belong to families with high cancer incidence and/or suffer from early onset tumours or multiple or multifocal malignancies. Many cases of hereditary predisposition to cancer are due to a germline mutation in one of the tumour suppressor genes. Some familial cancer syndromes show predisposition to a particular type of cancer (e.g. breast or colon cancer). The much rarer Li-Fraumeni syndrome (LFS) is distinct because members of LFS families suffer from a wide spectrum of different malignancies including sarcomas, brain tumours, breast cancer, adrenocortical carcinomas and other tumours. The cancer predisposition in most of these families is due to a germline mutation in the TP53 gene. It is generally expected that the tumours in most of such predisposed persons arise after the wild type TP53 allele is lost in a particular cell clone in a carrier individual. We show on our material that many tumours from germline TP53 mutation carriers retain the wild type TP53 allele. The development of these tumours in LFS individuals must therefore be based on another mechanism of the TP53 gene silencing than simple DNA loss. Alternatively, one functional TP53 allele may still be present in these tumours. We also compare these observations with records in our web database of published germline TP53 mutations, which is a very useful tool for different analyses of this intriguing syndrome. Supported by grant IGA MZ CR NC/6513-3.

P0074. Gene expression following tet-regulated reexpression of wt p53 in lung cancer cells

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The tumor suppressor p53 is inactivated in a wide range of human tumors. In non-small cell lung carcinoma (NSCLC) cell line NCI-H358 p53 and p16INK4a/p15INK4b are deficient while Rb is expressed. This condition occurs frequently in native human NSCLC and, therefore, appears to be particularly suited for studying the effects of reexpression of wild-type (wt) p53 in lung cancer cells. We generated the wt p53 inducible NSCLC line H358B22 using a tetracycline/doxycline-regulated (tet-on) expression system. High-level reexpression of wt p53 suppressed proliferation of H358B22 cells completely. Most growth arrested cells stayed viable over a period of 1 week. Therefore, p53 appears to function mainly as an inducer of cell cycle arrest rather than as an inducer of apoptosis in these cells. This growth inhibitory effect of wt p53 is reversible after 24 h of p53 induction, but it becomes irreversible after 48 h of wt p53 induction followed by resilencing of the exogenous wt p53. Therefore, genes regulated in growth arrested H358B22 cells were investigated by microarrays (Affymetrix), RT-PCR and Western blotting. Most differences in gene expression were reversible upon resilencing of exogenous wt p53. However, in irreversibly arrested H358B22 cells a subset of genes including Bax, Fas, p27KIP1, p21WAF1, B-myb, cyclin A and IGF-BP3 escaped reversibility.

P0075. GSTM1 null, GSTT1 null, GSTP1 (Ile105Val) and CYPA1 (T6235C) Genotypes in Childhood Acute Leukemia

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The purpose of the present study is to elucidate the role of GSTM1 null, GSTT1 null, GSTP1 (Ile105Val) and CYPA1 (T6235C) polymorphisms in the etiology of childhood acute leukemia. The study showed that: A) Frequencies of the genotypes were almost identical in 145 ALL patients and 186 healthy controls. Differences in the frequencies were not statistically significant in all genotypes (>p 0.2). The frequency of GSTM1 and GSTT1 double null genotype was lower in ALL (9.9%) than controls (13%). In ALL patients: 1- No statistically significant differences were found in the frequencies of genotypes between patients belonging to B cell (73) and non B cell lineage (41), yet the frequency of GSTT1 genotype was lower in the group non-B cell (17%) than B cell and control (23%). 2- No differences were found in frequencies of the genotypes between male and female patients. 3- There was no differences in distribution of

the genotypes among age groups, except frequency of the GSTT1 genotype was lower in patients 10-17 years (17%) than 0-2, 2-9 years (23%) and control. 4- The frequency of CYPA1 polymorphism was statistically significant in group of patients with WBC count 10.000-50.000 at presentation (58%) than <10.000 (20%), >50.000 (21%) (p 0.01) and control (29%). Frequency of GSTT1 genotype was lower in patients with >50.000 (10%) than others and control (23%). B) Statistically significant association was found in the frequencies of GSTT1 genotype between AML patients (3.4%) and control (23%) (p 0.016) while no association was observed for other genotypes.

P0076. Increased accuracy of leukemia diagnosis by combined analysis of morphology and FISH using the Duet automatic cell scanning system.

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Fluorescence in situ hybridization (FISH) is a valuable tool in clinical practice of leukemia. However, high false positive and false negative rates complicate the interpretation of results. These limitations are especially important in follow-up examinations, and in detection of minimal residual disease (MRD). Recently, the Duet scanning system (BioView Ltd, Rehovot, Israel) was introduced. The system provides two important features: Automatic scanning of large number of cells, and combined analysis of morphology and FISH on the same cells. Prior to scanning, blood samples are processed according to a unique protocol, which allows removal of RBC and 2 consecutive staining of WBC (giemsa or immunocytochemistry and FISH). This approach was applied to 80 samples of various hematological malignancies in order to: a) Determine the lineage of cells carrying specific chromosomal rearrangement. b) Enhance FISH analysis accuracy in leukemic cells. c) Determine clonality and maturity of residual recipient/donor cells in bone marrow transplantation. d) Determine the maturity of cells carrying chromosomal rearrangements in MRD. The results were compared to the diagnosis given by routine methods. We found that the Duet system enabled increased specificity and sensitivity of leukemic cells detection. Scanning automatically large numbers of cells provided rapid and efficient identification of rare cells in MRD cases (up to one leukemic cells in 15,000 WBC). The combined morphologic and FISH information of suspected cells enhanced the specificity of leukemic cells detection and reduced FP drawbacks. These preliminary results indicate the advantage of using such approach in diagnosis of leukemic diseases.

P0077. Are Fanconi Anaemia Genes Inactivated in Sporadic Acute Myeloid Leukemia?

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Fanconi Anaemia (FA) is an autosomal recessive disorder characterised by congenital abnormalities, defective haemopoesis and a greatly increased risk of Acute Myeloid Leukaemia (AML). We are investigating whether mutations in the FA genes might predispose to the development of sporadic AML. Quantitative fluorescent PCR was used to screen archival DNA from peripheral blood or bone marrow from AML cases for deletions in the cloned FA genes, *FANCA, C, D2, E, F, G*. Of the 103 samples successfully screened for the *FANCA* gene, four heterozygous deletions were found (see table). Sequence analysis of the other allele in these four cases did not locate a second mutation. A sodium bisulphite conversion assay was developed to detect methylation of the *FANCA* CpG island. There was no evidence of allele inactivation by hypermethylation in these 4 samples, nor in a further 28 non-deleted samples. No deletions were found on screening the *FANCC*, *D2*, *E*, *F* and *G* genes in 64, 68, 31, 42 and 51 samples respectively. *FANCA* is a large gene (43 exons, 80kb) with a high incidence of deletion mutations in affected FA patients. These results show that such deletions may also be found in sporadic AML and may have contributed to leukaemogenesis.

Charcteristics of FANCA deletion samples (*= deletions endpoints undefined)

Sample	Type	Cytogenetics	*FANCA* Deletion
1	Male 65 yrs FAB M2	43,XY,del(5)(q15q3?3),-6,-7, r(7),i(8)q),add(16)(q?24),-17,-22, +der(?)t(?;6)(?;?p11)	heterozygous ex5-43*
2	Female 45 yrs FAB M1	44.XX,add(2)(q2),add(5)(q?),-7, +?10, -12, add(16)(q2), -18, -20, +mar[4]	heterozygous ex19-21
3	Male 56 yrs FAB M6	44,XY,del(1)(q21q25),add(4)(q?25),-5,-7,-11,-12,del(12)(q21q24),add(13)(q13), add(16)	heterozygous 11-21
4	Female 69 yrs	N/A	heterozygous ex 5-43*

P0078. Gene expression patterns in childhood acute lymphoblastic leukemia

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Array hybridization technique represents a useful method for the expression profiling of large gene sets during disease processes. Using this technology we studied gene expression in childhood acute lymphoblastic leukemia (ALL) patients. For detection of transcription activity we used Human Cancer cDNA Nylon Arrays (Clontech, USA) with 588 genes that can be involved in transformation. Total RNA was isolated from bone marrow leukocytes of patients at the time of diagnosis (previously untreated). The standard sample was prepared by RNA mixing of control individuals (bone marrow donors). Our objectives were to identify genes that were differentially expressed in ALL and might contribute to the disease development (and characterization).

Obtained gene expression profiles of patients were compared with the standard profile. The majority of patient genes showed the similar gene activity as in the control sample (e.g. gluthatione-S-transferase homolog, vimentin, rho-GAP hematopoietic protein C1, rho GDP dissociation inhibitor 2, fau etc.). Many genes displayed significant expression changes only in some patients. In a few genes it was possible to observe similar significant changes of gene expression in most patients (eg. PCNA, MMP8). These changes might be associated with common stream of the disease process and they can be studied in more detail. Supported by the grant IGA MZ CR no. NM/5901-3.

P0079. The relationship between the chromosomal rearrangement complexity and disease agresivity in some cases of leukemia

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Among over 100 patients with myeloid and lymphoid leukemias investigated cytogeneticaly during the last 15 months, in four cases, disease evolution was determined by the complexity and nature of chromosomal abnormalities identified at the first presentation. First case, a 22 years old man with L3type ALL, exhibited: del 3q26;del 5p13; t(8;14)(q24;q13);del 9p11q11 and inv 15p12qter in all cells from bone marrow. He died after four months. The second case, a woman of 62 years old with acute leukemia weak-differentiated, refractory to treatment, showed 48-54 chromosomes and 3-4 markers derived from chromosomes 5 and 12. She died in the next three weekes. The third case, a young man of 27 years old, with acute myeloid leukemia, apart of Ph chromosome presented del11q21 and del16q22. The rapid death of the three cases was a powerful prove of positive correlation between the complexity of chromosomal changes and disease agresivity. In change, a constitutional translocation t(3;5)(q26;q21) identified in a 72 years old woman with ET, conferred favourable evolution of the disease after a succesfull treatment with HU. So, we appreciate that, if in the first three cases of myeloid

and lymphoid leukemias could be a direct relationship between the complexity of genomic rearrangements identified at the onset and agresive development of the disease, in the fourth case of ET, constitutional translocation t(3;5), seems to be not involved in the etiology of the disease.
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P0080. Expression of Negative Regulators of Cell Cycle in Human Acute Leukemia Cells.

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The negative regulators of cell cycle like cyclin dependent kinases inhibitors genes, Rb family genes and p53 gene play important role as inhibitors of cell proliferation. Incorrect expression of these genes may cause disturbances in cell machinery, uncontrolled cell division and consequently malignant transformation.In our research we examined the level of cell cycle negative regulators genes expression on mRNA level in bone marrow samples in patients with acute leukemia before treatment.For detection of mRNA we used the Multi Probe RNase protection Assay System (RiboQuant). We analyzed the expression of cyclin dependent kinases genes (p16 and p21 family), Rb family genes and p53 gene.Obtained results show significantly high level of p53, p27, p19 and p18 mRNA, while the level of Rb and p16 mRNA is very low in the examined cells.The correlation of the results with the level of other cell cycle regulators expressions and clinical data may give us important information about new prognostic factors in hematological malignancies.

P0081. Submicroscopic deletion at the breakpoint in chromosome der(9) in Ph+ acute lymphoblastic leukemia (ALL)

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Submicroscopic deletions in the breakpoint region of chromosome der(9)t(9;22) are found in ~25% of patients with chronic myeloid leukemia (CML). Notably, these deletions are strongly associated with a shorter life expectancy when non transplanted CML patients are compared. We present molecular and clinical data of a 22 year old male patient with a t(9;22) positive B-cell specific acute lymphoblastic leukemia (B-ALL) that exhibit a deletion in chromosome der(9). In ALL this deletion was first and uniquely detected so far in one of 48 ALL patients investigated by Reid et al (abstract: 1334, ASH meeting 2001). The rare occurrence of this deletion in ALL makes it difficult to evaluate the clinical impact. The deletion we found is located proximal to the breakpoint in der(9)t(9;22). RT-PCR detected a b3a2 BCR-ABL and failed to detect an ABL-BCR transcript. No response to therapy was achieved with a high dose protocol (Hölzer Studie 5/93). The patient was subjected to salvage therapy with Idarubicin/AraC and reached a partial remission with 20-25% BCR-ABL positive cells at day 125 after initial therapy. At present, STI571 is administered and a stem cell donor is searched. The data confirm that deletions in der(9) can also be found in Ph+ ALL. The clinical significance of this rare deletion in ALL is unknown and has to be evaluated by increasing the study cohort.

P0082. Acute monocytic leukemia and multiple abnormalities in a child with duplication of 1q detected by GTG-banding and SKY.

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Patients with 1q duplication have demonstrated a wide range of multiple congenital abnormalities, but a clinical delineation of a trisomy 1q “syndrome” was proposed. Alterations involving this same chromosomal region have also being described in various hematopoietic malignant disorders and a series of candidate genes that may be associated with neoplasia have been described in this region. We describe a female girl with low birth weight, microcephaly, mid facial hipoplasia, synophris, short palpebral fissures, epicanthic fold, beak-like nose, narrow palate, teeth abnormalities, cardiac defect, syndactily, and motor delay, that presented, at 18 months of age, an acute monocytic leukemia (FAB-M5) according to cytological, histochemical and immunophenotyping features. The patient failed to achieve remission, and died 2 months after diagnosis. Cytogenetic study of the bone marrow cells by GTG-banding showed: 44~48,XX,-X[9],dup(1)(q23q44)[35],+2[27],-6[13],+7[4],-8[3],-9[7],+9[2],+10[3],+11[13], +12[5],-14[5],-15[4],-17[7],-18[13],-19[3],+21[5],-22[5],+22[2],+mar[5]cp[35].Spectral karyotyping (SKY) was also performed to identify the aberrations 46,XX,der(1)dup(1)(q23q44)t(1;1)(p36;q32),der(6)t(6;8)(p25;q13),+11,der(11)t(11;18)(q10;q10).Peripheral blood cytogenetic analysis was not performed due to repeated blood products transfusions and the precocious patient death. The dismorphological features with the dup(1q) founded in all bone marrow cells analyzed suggest that this is probably a constitutional chromosome alteration and the first, in our knowledge, association of a trisomy 1q”syndrome” with AML.
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P0083. Three new cases of complex Ph’ variants in Chronic Myeloid Leukemia

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A 90-95% of patients diagnosed with Chronic Myeloid Leukemia (CML) show the Philadelphia chromosome (Ph) as a result of the t(9;22)(q34;q11). About 5-10% of CML show the variant forms: simple (22q11qter is translocated into a chromosome other than 9) and complex (three or more chromosomes are involved).
We present cytogenetic, fluorescence in situ hybridization (FISH), and molecular analyses in three cases of the complex variant.
The chromosome bands involved were 11q13 (two cases) and 1p36.1. The FISH analyses (locus specific, centromeric and whole chromosome painting) showed that the bcr/abl fusion gene was in chromosome 22 in all three cases, suggesting a complex variant rather than a clonal evolution.

P0084. Characterisation of Acute Myeloid Leukemias (AML) with complex aberrant karyotype using gene expression analysis and mutation screening of candidate genes

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AML represent a pathogenetically and prognostically heterogeneous group. For differentiation of AML-subgroups, cytogenetics offers the most evaluated and established criteria today. 10-15% of AML-patients show complex aberrant karyotypes in leukemic blasts, associated with a most adverse progression of the disease. So far, no crucial candidate genes relevant for the pathogenesis were identified. Data obtained by 24color-FISH and CGH in 50 AML-cases with complex aberrant karyotype demonstrated a much larger percentage of loss than gain of genetic material. Frequent observations included deletions of the entire chromosomes 5 and 7, as well as interstitial deletions within their long arms.
These deletions may represent the first of two required mutation events to deplete a tumorsuppressor gene’s function. Alternatively, haploinsufficiency with only one mutation event might already be sufficient to reduce the physiologically necessary amount of gene product.
To evaluate both models, we currently investigate 25 of these AML-cases with complex aberrant karyotype more precisely on molecular genetic level: utilizing gene expression analysis (GeneChip U133) and mutation screening (Single Strand Conformation Polymorphism Analysis, SSCPA) we focus on 20 functionally relevant candidate genes on chromosomes 5 and 7, involved in apoptosis, cell cycle/transcription regulation and DNA repair mechanisms.
So far, SSCPA in 25 AML-patients and 10 healthy controls using different conditions excluded the *General Transciption Factor*

GTF2H2 (5q12.2-q13.3), involved in transcription/transcription-mediated DNA-repair, as a major candidate gene in complex AML-pathogenesis.
Our future investigations aim at providing a deeper insight into basic pathogenetic mechanisms of complex AML with subsequent implementation in prognosis and therapy strategy.

P0085. Validation of human BAC clone microarray based CGH studies in HL-60 cell line.

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Comparative genomic hybridization (CGH) is a method, used to detect, and map DNA sequence copy number differences between two genomes in a single experiment. Although it is a valuable technique, the lower resolution of CGH compared to molecular genetic techniques have limited its application. The utilization of microarray technology in CGH analysis has the potential to meet these challenges. For this purpose, a well-characterized promyelocytic cell line HL-60 was analyzed by using Human BAC Array- 3MB system, which was developed by Spectral Genomics™. The glass array is composed of 1003 non-overlapping BAC library clones which encompass the human genome with a resolution of 3 Megabases. In HL-60, amplification of the region of 8q24, an extra copy of chromosome 18, and deletions in loci of 5q11.2-q31, 6q12, 8p23, 9p21.3-p22, 10p12-15, 14q22-q31, 16q21, and 17p12-17p13.3 were detected. These results are largely concordant with the previously reported aberrations, and indicate that this system might be an alternative to conventional CGH analysis.

P0086. High-throughput tissue microarray analysis of 11q13 genes amplification (CCND1, FGF3/FGF4, FGF3, EMS1) in urinary bladder cancer

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Gene amplification is a common mechanism for oncogene overexpression. High-level amplifications at 11q13 had been repeatedly found in bladder cancer by comparative genomic hybridization and by other techniques. Putitative candidate oncogenes located in this region are CCND1, EMS1, FGF3 and FGF4. To evaluate the involvement of these genes in bladder cancer, we screened a tissue microarray (TMA) containing 2317 samples by FISH. Among all tumors with 11q13 amplifications (13.3%) 68.3% had all 4 genes amplified, 19.5% had amplified CCND1, FGF4 and FGF3 together, 0.8% had FGF4, FGF3 and EMS1 coamplified. Single amplification of CCND1 was found in 9% of the tumors, while 1,6% had single amplification of EMS1 and 0.8% - of FGF4 suggesting that CCND1 is the major target gene in the 11q13 amplicon in bladder cancer. The frequency of both gains and amplifications of all genes increased significantly from stage pTa to pT1-4 and from low to high grade tumors. Increased copy number changes of all 4 genes were associated with survival of patients with tumors from all stages and progression of pT1 tumors and were not associated with recurrence of pTa tumors and survival of patients with pT2-4 tumors. Tumors with gains of FGF3, FGF3/FGF4 and EMS1 were shown to behave like tumors with amplifications rather than like normal tumors contrary to CCND1 where gained tumors behaved like CCND1 normal rather than like CCND1 amplified tumors.

P0087. Dystrophic Scoliosis And Genetic Polymorphisms In Patients With Neurofibromatosis

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The dystrophic form of scoliosis in neurofibromatosis 1 (NF1) is often associated with severe decrease in bone mineral density, significantly hindering reconstructive bone surgery. Osteoporosis has been found to be associated with distinct polymorphisms of the vitamin D receptor gene (VDR), oestrogen receptor gene (OER) and the collagen 1α1 gene (COL1A1) in the general population. The purpose of the present case-control study was to evaluate the hypothesis, whether the genotypes at these three polymorphic loci are associated with decreased bone mineral density in scoliotic patients with neurofibromatosis. Genotype of 21 selected NF1 patients with scoliosis and decreased bone mineral density was compared to 21 patients with non-scoliotic NF1 with normal bone density. Patients with idiopathic scoliosis with normal bone density measurements (21) have been also assessed for the same genetic polymorphisms. In this pilot study of altogether 63 individuals no association was found between VDR and OER polymorphisms, and the phenotype. The genetic marker distribution in the idiopathic scoliosis (IS) group did not significantly differ from that of scoliotic NF1 patients. However, we observed a threefold prevalence of the homozygous polymorphism (CC) over the heterozygous form (Cc) of the COL1A1 gene in non-scoliotic NF1 patients compared to patients with scoliosis presenting with an almost equal distribution in this genotype. This difference was not statistically significant. The sample size of this pilot study is not large enough to draw a final conclusion. A possible protective role of CC genotype in non-scoliotic NF patients deserves reevaluation in a larger group of patients.

P0088. Radiological appearance of intracranial tumours in neurofibromatosis (NF1)

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In a population-based study on neuroradiological imaging of individuals with NF1, 10 of 124 studied patients (8%) presented with intracranial tumours other than optic gliomas or T2 hyperintense lesions. Six patients aged 6 to 53 years had an astrocytoma, one patient had a suspected astrocytoma which proved histologically to be normal brain tissue, one patient had a small lipoma in the interpeduncular cistern, one patient had a hypophyseal adenoma and one patient presented with an enhancing lesion of the cavernous sinus. - The astrocytomas showed wide variation in their behaviour and MR imaging. Four of the tumours were progressive, one histologically confirmed astrocytoma disappeared spontaneously and one astrocytoma appeared within a previously detected T2 hyperintense lesion, which was partly located in the region of the optic radiation and had remained stable for several years. The results indicate that the fate of an astrocytoma in NF1 cannot be predicted on the basis of one imaging only, but that the patients need close follow-up.

P0089. Do some additional chromosome rearrangements mean a favourable T-cell prolymphocytic leukemia prognosis with preserved alkylator based treatment sensitivity?

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A 77-year-old woman came to haematological department because of leucocytosis, (WBC 276x109/L; Hgb 111 g/L, Plt 239x109/L), sweating and weight loss. Bone marrow biopsy revealed 80% lymphoid-cell infiltration. Morphologically cells appeared as prolymphocytes and immunohistochemically they were CD3, CD4, CD5 positive, while being CD8, CD20, CD30, CD43, CD56, TIA-1 and Granzyme B negative. Flow-cytometrically performed T-lymphoid immunological markers CD2, CD3, CD4, CD5 and CD7 were highly, more than 90% positive. Diagnosis of T-cell prolymphocytic leukemia (T-PLL) was made and the later was confirmed also by cytogenetic analysis. T-PLL specific chromosome rearrangements were observed: inv(14)(q11q32), i(8)(q10) and del(11)(q22q23) involving ATM gene. Complex translocation of X chromosome, probably involving MTCP1 gene, was present on der(X)t(X;3)(q28;p25)t(X;16)(p14;q12). There were several other chromosome rearrangements observed, including chromosomes 5, 6, 13, 14, 17, 20 and 22. Classical cytogenetic analysis was confirmed by FISH, using Cytocell Octochrome Multiprobe System, and some locus specific DNA probes.

T-PL leukemia is aggressive, and refractory to alkylator-based therapy, with a median survival of 7 months. Treatment options are highly immunosupressive 2-CDA, Pentostatin or Campath 1-H. Because of the patient's advanced age, we have started with chlorambucil 10 mg/m2 for 5 consecutive days, repeatingly every 4 weeks. After 4 cycles of chlorambucil the patient was without any clinical symptoms, with WBC 23,4x109/L, Hgb 128g/L, Plt 256x109/L. To our knowledge, the additional chromosomal rearrangements: der(6)t(X;6)(p14;q25), der(13)t(13;14)(q22;q11), t(5;13)(q34;p11), r(17)(p13q21), t(17;20)(q21;q13), 22p+ , were not yet described in the literature. Some of them may means favourable T-PL prognosis because of preserved alkylator agent treatment sensitivity.

P0090. Prognostic Significance Of Small Cell Clones With Hyperdiploidy In Childhood Acute Lymphoblastic Leukemia (all).

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Children with ALL and chromosome hyperdiploidy in bone marrow cells have a better prognosis in contrast to those with other cytogenetic abnormalities. Therefore early detection of hyperdiploid cell clones is important and can lead to appropriate less aggressive therapy protocol with the lower risk of the late side effect. For the assessment of hyperdiploidy we use consecutive double target interphase FISH (I-FISH) with combination of alpha-satellite and/or locus-specific probes for 10 chromosomes most frequently overrepresented in hyperdiploid clones (200 interphase nuclei analysed per slide and probe-mix, cut-off level 2.5% tested on controls, standard deviation not exceed 0.5%). I-FISH is quick and sensitive screening method which enables to find even "hidden" small pathological clones. Structural and/or complex chromosomal aberrations in hyperdiploid cells were analysed by multi-color FISH (mFISH).
During the last four years we examined prospectively or retrospectively 88 children with ALL (56 boys, 32 girls; mean age 8 years). Various level of hyperdiploidy was found in 60 children (68%). The extent of pathological clones being 2.5 - 100%. Small clones under 10% were detected in 18 patients (20,5%). Structural or complex chromosomal rearrangements together with hyperdiploidy were found in 23 patients (26%). We compare FISH and cytogenetic findings, results of DNA analysis by flow cytometry and clinical course of the disease in all patients with a particular respect to the prognostic significance of small pathological clones and complex chromosomal rearrangements.
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P0091. Cytogenetic studies in T-cell acute lymphoblastic leukemia: a report of 35 cases.

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A total of 198 patients with acute lymphoblastic leukemia (ALL), including 189 at diagnosis and 9 at relapse, had a cytogenetic analysis on a bone marrow sample between 1981 and 2001. Thirty-five ALL (17.7%) were of the T cell lineage. The immunophenotyping performed on 32 cases showed that 30 were true T-cell ALL, one was mixed T-cell/B-cell and one mixed T-cell/Myeloid. The 35 patients were distributed in 14 children and 21 adults. The quality of the chromosomal preparations was too poor to allow a feasible identification in 2 cases and one culture did not yield metaphases. Karyotyping was sucessfully performed in 32 patients. A normal karyotype was observed in 5 of the 13 pediatric cases (38.5%) and in 5 of the 19 adult cases (26.3%). These values are within the range observed in other series of T cell-ALL. Numerical chromosome abnormalities were rare, 77.3% of the abnormal karyotypes (17/22) being pseudodiploid. Translocations involving band 14q11 were observed in 7 patients whereas band 12p13 was deleted in 2 cases and translocated in a further 3 cases. The short arm of chromosome 11 was involved in 4 translocations, band 11p13 in 2 and band 11p15 in another 2 cases [t(4;11) and t(1;4;11)]. Other recurring structural rearrangements include del(6q) in 3 cases and del(5q) in 2. Most of these recurrent abnormalities are different from those of B-lineage ALL. Some are known to involve T cell receptor genes whereas others can lead to the discovery of new genes that are important to T-lineage leukemogenesis.

P0092. Detection of Philadelphia Chromosome in Chronic Myelogenous and Acute Lymphoblastic Leukemia in two locations in Ecuador.

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A previous study sustained that there may be a difference in the presence in Philadephia (Ph) Chromosome t(9;22) in the studied ecuadorian series due to the ethnical content and geographical location (Quito, 2800m) of the studied human group.
We present here data that supports that there is no influence of these aspect in the presence of this chromosomal marker in cases of Chronic Myelogenous and Acute Lymphoblastic Leukemias (CML and ALL, respectively) in Ecuador.
The study was performed in two major laboratories in Guayaquil (at the sea level) and Quito (2800 m over the sea level) and included a population with varied ethnical content. The described cases correspond to ALL and CML diagnosed by bone marrow smears and Immunocytochemistry, this are 199 cases of ALL and 295 cases of CML studied in both cities.
The CML presented and average frequency of Ph+ of 85%, and the ALL cases had a frequency of 14%.
No statistical difference in the presence of Ph Chromosome in neither CML or ALL was found in the studied cases at the coast area in relation to the frequencies published for Quito and to the world statistics, although in a previous publication of the molecular analysis of Quito's cases it was shown the presence of a particular pattern of the *abl-bcr* rearrangements.

P0093. Molecular and cytogenetic changes in STI571 (Gleevec) treated Acute Lymphoblastic Ph + Leukemia

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Targeting the tyrosine kines activity of Bcr-Ablоncoprotein is effective therapeuitc option of Ph-chromosome positive CML and ALL. However, accumulating clinical experience describes emerging tumour resistance to STI571 tyrosine kinase inhibitor in the treatment course.
A 36-year old woman with relapsing Philadelphia positive Acute Lymphoblastic leukemia (Ph+ALL) was treated with STI571 (600 mg/d). After 3 months haematological response in terms of correcting leukopenia, reducing the number of immature cells in the bone marrow by 50% and decreasing the need for platenet and haemoglobin transfusion was seen. However, in the sixth month of treatment patient stopped responding to the drug with rapidly incresing number of blasts. At that point standard G-banding of leukaemic cells identifed additional chromosomal changes:del(6q) and t(11;14)(q13;q32). Molecurarly, we were able to detect Major and Minor-breakpoint Bcr gene rearrangements in the fusion with the Abl gene while molecular cytogenetics showed amplification of the Bcr-Abl gene detected as the emergence of an extra Bcr-Abl gene copy in 17%of cells.PCR amplification of the BCL1-IgH fusion gene was negative and there was no BCL1 expression by the blasts (immunocytochemistry). Biological mechanisms of these genetic events are unknown and multiplication of Philadelphia chromosome can be related to the acquired.

P0094. CGH In The Evaluation Of The Placenta In Abnormal Pregnancies

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Confined placental mosaicism (CPM) in term placental tissues is usually diagnosed by conventional cytogenetic analysis and more recently by fluorescence in situ hybridization (FISH) of the trophoblast. In this study, we describe the use of comparative

genomic hybridization (CGH) for detection of chromosomal aneuploidy in 26 fresh and 14 paraffin embedded placentas and evaluate the sensitivity of this novel approach for CPM diagnosis in multiple placental samples.
We applied CGH technique to samples taken from various sites of placentas originating from abnormal pregnancies (23 IUGRs, one with fetal malformation, one with toxemia, one with hydrocephalus and 2 undetectable MSAFP). In the control cases (7 normal and 5 with known aneuploidy) CGH concurred with the known karyotype. The most common aberration in the IUGR cases was the addition of a whole or part of X chromosome. Other aberrations such as addition of Y chromosome , addition of 13(q22) and loss of chromosome 17 where found in other cases. There was also one IUGR case of trisomy 8 (in one site) and 47,XXY found in all sites. In the two cases with the MSAFP=O monosomy 16 was detected (in one case on both sites searched). Some of the results were confirmed by the FISH technique.
Our results demonstrate the usefulness of CGH technique in the genetic evaluation of fresh and paraffin embedded placentas in problematic pregnancies even when its morphology is normal.

P0095. Detection of chromosomal aneuploidy in spontaneous abortions using comparative genomic hybridization (CGH)

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Chromosomal aneuploidy is a common cause of abnormal prenatal development. Comparative genomic hybridization (CGH) provides a rapid and comprehensive detection chromosomal gains and losses in the test genome and maps the aneuploidies onto normal metaphase chromosomes. Among the 52 tissue culture of spontaneous abortions, 10 cases showed failure of fetal cell growth in culture and could not be identified reliably by conventional cytogenetics. CGH analysis was successfully performed for detection of chromosomal aneuploidy in spontaneously aborted specimens with tissue culture failure. Balanced karyotype profiles were obtained for 5 samples. All of them were analysed by fluorescence in situ hybridization (FISH) with centromere-specific DNA probes to exclude polyploidy. Nothing cells with abnormal level of ploidy were found. Five spontaneous abortions (50%) have monosomy 22 and trisomy 10, 14, 18 and 21. Monosomy 22 identified by CGH is one of the most rare aneuploidies in spontaneous abortions. In all cases with an indication of chromosomal imbalance by CGH, FISH with chromosome-specific DNA probe was performed to confirm the presence of aneuploidy. As determined by FISH analysis two cases with trisomy 10 and monosomy 22 were mosaics with frequency of abnormal cell line 68% and 33% respectively. Advantages and limitations of CGH for a detection of complete and mosaic forms of aneuploidy are discussed.

P0096. Marker chromosome identification with chromosome microdissection and reverse FISH and CGH

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Reverse painting fluorescent in situ hybridization (FISH) on the normal metaphase with probes generated by chromosome microdissection and comparative genomic hybridization (CGH) are powerful methods to identify the origin of marker chromosomes. Three cases having unidentified marker chromosomes were studied by reverse painting FISH and CGH. Reverse FISH probes were generated from five copies of each marker chromosomes dissected with micromanipulator, amplified with DOP-PCR, and labeled with fluorochromes. The probes were hybridized to normal metaphases and the origin of marker chromosomes could be determined. Three marker chromosomes were identified as derivative chromosome 15 inducing partial trisomy of 15q, duplication of the short arm of chromosome 17 and duplication of the short arm and deletion of the part of the long arm of chromosome X. CGH showed concordant results with reverse FISH.

P0097. Reexamination of chromosome 2 rearrangements characterized by multicolor banding (MCB) by region-specific FISH probes

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Conventional banding techniques often fail to characterize the exact nature of chromosomal rearrangements. The MCB technique has demonstrated to improve the definition of chromosomal breakpoints (e.g. Starke et al., 2001, PrenatDiag, 21, 1049-1052, Dufke et al., 2001, Europ J Hum Genet 9, 572-576). Here MCB was applied to identify human chromosome 2 breakpoints in two clinical cases. To show how precise the correlation between the MCB pseudocolors and the GTG banding works the results of MCB were reexamined using region-specific YAC or BAC probes. The chosen band resolution of chromosome 2 was 400 bands per haploid karyotype. Case 1 presented with primary mental retardation and severe delayed speech development. The boy had a der(9)t(2;9)(2q24.2;9 p11.2) according to GTG banding. MCB showed, however, that the translocation was balanced although it seemed to be not according to GTG banding; new karyptype: t(2;9)(q24.2;p24.3). Case 2 showed primary mental retardation, tendency to seizures, craniofacial dysmorphisms and adipositas. The type of aberration in this male patient could not be defined by GTG-banding (inv(2)(p11q23)+dup?o r.inv(2)(p21q24.1)+del?). MCB could characterize the rearrangement as inv(2)(p15q24.3). In both cases the results of MCB were confirmed with a panel of region specific YAC/BAC probes. In all 20 MCB metaphases analyzed per case the breakpoints appeared within the same pseudo-colored bands. Thus, the highly reproducible MCB pattern, can be used to characterize abnormalities that remain cryptic or unresolvable in G-banding analysis. Supported by DFG (436 RUS 17/40/00; PO284/6-1), Wilhelm Sander-Stiftung (99.105.1), the EU (ICA2-CT-2000-10012 and QLRT-1999-31590). Dr. Rocchi (Bari, Italy) is acknowledged for YAC/BACs.

P0098. Identification of satellite sequences in metaphase and interphase with peptide nucleic acid (PNA) probes using multicolor fluorescence *in situ* hybridization.

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Multiplex fluorescence in *situ* hybridization (M-FISH) can be used to detect marker chromosomes, chromosomal rearrangements in cancer, prenatal diagnosis etc. Regular M-FISH requires a large amount of labeled DNA, the hybridization time is longer and is less informative in interphase nuclei compared to standard FISH. We have designed and developed directly labeled PNA probes to distinguish up to 2^n-1 chromosomes (where n is the number of different fluorochromes) using an epifluorescence microscope equipped with a digital imaging camera and computer software for pseudocoloring and merging images. Peptide nucleic acids (PNA) are synthetic mimics of DNA in which the phosphodiester backbone has been replaced with 2-aminoethyl glycine linkages, but maintaining the four natural nucleobases. PNA probes bind to the complementary DNA sequence obeying Watson-Crick base pairing, however the neutral backbone of the PNA molecule allows for the PNA/DNA binding to occur more rapidly and more tightly than DNA/DNA binding. Chromosome specific composite PNA probe sets were generated from the human satellite sequences, in which the different fluorochromes were incorporated to address specific issues, like identification of marker chromosomes and anueploidies. With four fluorophores, we were able to enumerate up to 15 chromosomes in both metaphase spreads and interphase nuclei in a single hybridization experiment. Our data suggests that multiplex fluorescence in situ hybridization (M-FISH) using PNA probes could have wide clinical utility, particularly in detection and enumeration of chromosomes in a given sample. Multi-parameter hybridization analysis should facilitate the study in molecular cytogenetics and probe-based diagnosis of pathogens.

P0099. Multicolor fluorescent in situ hybridization in neuronal cells as an approach for identification of low level chromosomal aneuploidy in the brain

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Fluorescence in situ hybridization (FISH) of DNA-DNA or DNA-RNA

using post-mortem brain samples is an approach to study a low-level chromosomal aneuploidy and selective expression of specific genes in brain of patients with neuropsychiatric diseases. We have performed a pilot molecular-cytogenetic analysis of post-mortem brain of schizophrenic patients. Multicolor FISH on two post-mortem brain samples of normal and six schizophrenic individuals (area 10 of cortex) was applied. A set of DNA probes for FISH included (i) centromeric alphoid DNA probes for chromosomes 7, 8, 13 and 21, 18, X, Y;(ii) classical satellite DNA probes for chromosomes 1 and 16 and (iii) region-specific DNA probes for chromosomes 13, 21 and 22. Statistically significant level of aneuploidy (up to 3-4% of neurons) involving chromosome X and 18 was detected in two post-mortem brains of patients with schizophrenia. The multicolor FISH assay could be applied to study low level of chromosomal aneuploidy, intranuclear distribution and conformation of heterochromatin, abnormal patterns of chromosomal organization and functional gene expression in situ in post-mortem brain at many neurogenetic diseases. Schizophrenia and Rett syndrome are the diseases of special interest for extended molecular-cytogenetic analysis as both of them could suspect alterations in chromatin conformation and differential gene expression in brain cells. Supported by Copernicus 2 grant.

P0100. CGH contribution in the delineation of chromosomal rearrangements

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Comparative Genomic Hybridization (CGH) is able to identify the origin of extra or missing chromosome material when either the small size of the segment or a non-discriminatory banding pattern does not allow a cytogenetic diagnosis. It has also the potential to detect both terminal and interstitial rearrangements. We illustrate here the contribution of CGH in the delineation of 13 different cases studied in our laboratory. In most cases, CGH was performed to characterize a rearrangement detected using classical cytogenetic methods i.e identification of extra or missing chromosome material, confirmation of an imbalance or accurate definition of the chromosome breakpoints. In all these cases CGH was decisive. In some other cases, classical cytogenetics (550 to 850 bands) did not detect any chromosome imbalance when CGH detected a chromosome imbalance in several cases. All abnormal results were confirmed using whole chromosome painting and/or FISH with subtelomeric probes. In conclusion, CGH is a very powerful method to analyze an unbalanced rearrangement already identified using classical cytogenetics and requiring further characterization . When no chromosomal rearrangement is observed, CGH could be an alternative to multiprobe FISH study of all subtelomeric regions. However its use as a screening tool in unexplained mental retardation remains limited due to the difficulty of obtaining chromosomal preparations allowing high quality hybridizations on a regular basis.

P0101. The Impact of BRCA1/2 susceptibility genes on women's mental health

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Three predominant mutations in BRCA1/2 genes have been found in 3% of the Jewish Ashkenazi population. Such mutations significantly increase lifetime risk for developing breast and/or ovarian cancer. The present study focuses on the impact of being BRCA1/2 mutation carrier on women's mental health. A retrospective study was conducted in the oncogenetic clinic at Rambam medical center, Israel. One hundred and thirty eight women were recruited and evaluated regarding their medical history and mental health state using the BSI (The Brief Symptom Inventory; Derogatise 1982). All women were genotyped for BRCA1/2 founder mutations. Of the 138 women, 39 (28%) were mutation carriers. Breast cancer was diagnosed in 69 (50%) women. The mean age at diagnosis was 45.7±10.7 years and at the interview was 50±10.5 years. Univariate analysis of Variance (ANOVA) [Morbidity (with/without breast cancer) X Mutation (carrier/non-carrier)] revealed significant effect for morbidity, mutation, and the interaction on four sub-scales of the BSI and on its total score (GSI). Apparently, asymptomatic mutation carriers expressed the highest levels of somatization (F=30.0; p<.001), depression (F=9.1; p<.01), interpersonal sensitivity (F=4.5; p<.05) hostility (F=14.4; p<.001), and GSI (F=8.9; p<.01). It may be that healthy women who carry a mutation are more stressed regarding their health status than breast cancer mutation carriers.

P0102. Familial or sporadic? Unexpected results in the diagnosis of hereditary breast cancers

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Genetic counseling and risk assessment in families with breast/ovarian cancer is regularly based on pedigree analysis. However, familial and sporadic cases may occur in the same family. We report on three families with unexpected segregation of BRCA1/2 mutations in affected and unaffected family members.
Family No 1: The female proband, who presented with breast cancer at 28 years of age, carried the common T300G mutation. Surprisingly her mother, diagnosed with breast cancer at 33 years of age, was tested negative for this mutation. T300G was identified in the proband´s healthy father.
Family No 2: Three siblings (one man, two women) and their deceased father had been diagnosed with breast cancer. The brother and one sister, diagnosed at 40 years of age, carried the common 2041insA mutation in the BRCA2 gene. The other sister, diagnosed at 60 years of age, tested negative for this mutation.
Family No 3: The female proband presented with breast cancer at 37 years of age. She carried a novel BRCA1-splice mutation(4304+2in sAdel21bp). Her mother, diagnosed with breast cancer at 55 years, was tested negative, although she had two affected sisters. The proband`s healthy father however with an unremarkable family history carried the splice mutation. br />We conclude that for exact risk assessment and genetic counseling in the hereditary breast/ovarian cancer syndrome, each affected and unaffected family member at risk should be tested.

P0103. Familial Dissemination of BRCA1/BRCA2 Test Results

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Study Design: Retrospective follow up study of individuals who notified that they are carriers of a BRCA1/BRCA2 mutation. Participants received and returned by mail an assessment of the pattern of their disclosure of the results of their genetic testing in their family.
Instrumentation: Self-report questionnaire follow up assessment of mutation carriers who have received results.
Most probands (60%) were the first in their family to receive results, and almost half (47.3%) had agreements with family members prior to testing to disclose results. Probands with such agreements were more likely to have family members present during genetic counseling and results disclosure, C2 (1) = 4.0, p < .05. Individuals with such an agreement reported that a sense of obligation, encouragement from their physician and family members, and being asked by family members were stronger determinants of their decision to share results than did probands without a prior agreement (all ps < .001). Probands with such an agreement were more likely to endorse the following factors as facilitating disclosure: support from close family relationships, their physicians' support, concern that family members be able to use information to make healthcare decisions for themselves and their children, and being asked directly by family members (all p < .05). These data suggest that the family context is a crucial determinant of how genetic testing information is disseminated, and that interventions aimed at improving dissemination of genetic testing information need to focus on agreements to disseminate test results made prior to the receipt of results.

P0104. Genetic polymorphisms of biotransformation enzymes and susceptibility to breast cancer

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Breast cancer is the most common malignancy in women and second leading cause of death from cancer. The genetically variable

biotransformation enzymes: epoxide hydrolase (EPHX), NADPH-quinone oxidoreductase (NQO1), and glutathione S-transferases (GST's) metabolize drugs, carcinogens, and natural products. In addition, it is generally accepted that majority of human cancers results from exposure to environmental carcinogens. Considering the role in the metabolism of chemicals played by biotransformation enzymes, we aimed at determining whether any association exists between genetic polymorphisms of biotransformation enzymes and individual susceptibility to breast cancer in Czech women.
Genotyping analyses were performed by PCR-RFLP to determine the frequency of polymorphisms in EPHX (exons 3 and 4), GSTM1 (deletion), GSTP1 (exon 5), GSTT1 (deletion) and NQO1 (exon 6). The study population consisted of 169 breast cancer cases and 231 healthy controls.
No association between polymorphisms in EPHX, GSTM1, GSTP1, and GSTT1 and breast cancer was found. On the contrary, a significantly different distribution of genotypes in NQO1 between controls and breast cancer group was confirmed by chi-square test (P=0.003, chi-square=11.83, DF=2). We have observed significantly higher frequency of mutated genotype S/S in patients in comparison with controls (8.1% vs. 1.3%). Homozygous mutant genotype S/S leads to complete lack of activity NQO1. Moreover, the involvement of NQO1 in colorectal cancer and tumor resistance to anticancer drugs was implicated.
Our results suggest that NQO1 may be an important factor in susceptibility to breast cancer and its role should be further investigated.
This study was supported by grant GACR No.: 310/01/1537.

P0105. Mutational analysis of the Tuberous Sclerosis Complex (TSC) genes

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Tuberous Sclerosis Complex (TSC) is an autosomal dominantly inherited disorder characterized by development of benign tumours (hamartomas) in many organs. Hamartoma formation in the central nervous system is associated with some of the most problematic clinical manifestations of TSC, and can lead to intellectual handicap, epilepsy and autism. Inactivating mutations in either of two tumour supressor genes, TSC1 or TSC2, is the cause of this syndrome.
Here we have established a mutational analysis for TSC1 and TSC2. For the 21 coding exons of TSC1, we have developed a mutation identification assay that combines long-range PCR with automated sequencing. For mutation screening of the 41 coding exons of TSC2, we have developed a rapid and efficient denaturing gradient gel electrophoresis (DGGE) assay.
We are currently collecting DNA samples from Danish tuberous sclerosis patients. Presently, we are carrying out mutational analysis on DNA from peripheral blood from 25 Danish TSC patients (15 sporadic and 10 familial cases). Furthermore, Southern blot analyses using TSC1 and TSC2 cDNA probes are also carried out. Sofar, we have identified a total of 11 mutations, 4 of which have been identified previously in another lab and 9 of which are novel mutations. In one patient mosaicism was detected. A number of polymorphisms were also detected.

P0106. Neurofibromatosis-Noonan phenotype with a mutation (R816X) in the NF1 gene.

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An 11-year-old girl of Albanian origin was diagnosed to have neurofibromatosis 1, but she also had features of the Noonan syndrome including short stature (-4.2 SD), pulmonary valvular stenosis, shield chest, posteriorly rotated earlobes with thick helices, and high palate. The diagnosis of neurofibromatosis type 1 was made on the basis of several cafe-au-lait spots, axillary freckling, and bilateral Lisch noduli of the iris. The MRI studies showed both bright signals in left globus pallidus and hippocampus and a thick medulla oblongata. She had mild to moderate developmental delay.
In molecular genetic studies, a nonsense mutation (R816X) was identified in the NF1 gene. The parents and the 5 siblings had no signs of either neurofibromatosis 1 or the Noonan syndrome, and neither one of the parents carried the mutation.
Previously, Bahuau et al.(1998) found the same mutation in a family segregating both NF1 and Noonan syndrome. They suggested a coincidental occurrence of the two conditions, having evidence from their pedigree that both phenotypes co-localize and that another locus for Noonan syndrome resides on 17q in close vicinity of the NF1 gene. They also identified this mutation in 3/184 (1.6%) individuals with only classical NF1. However, the independent finding of the same R816X mutation in an unrelated patient displaying both phenotypes suggests that this particular mutation may bring about the expression of the Noonan phenotype in individuals with NF1.

P0107. Application of denaturing high-performance liquid chromatography-based analysis to neurofibromatosis type 1

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Neurofibromatosis type 1 (NF1; MIM# 162200) is a common autosomal dominant disorder, characterised by café-au-lait spots, peripheral neurofibromas, Lisch nodules and freckling. The high mutation rate at the NF1 locus results in a wide range of molecular abnormalities. The majority of NF1 mutations are private and rare, generating high allelic diversity with a restricted number of recurrent mutations. Denaturing high-performance liquid chromatography (DHPLC) has been recently introduced as a rapid and highly sensitive method for detecting sequence alterations, well suited to mutation detection. We have scanned 17 exons of the NF1 gene using DHPLC method in a series of 39 NF1 patients. Five novel mutations (496delGTTT, E725X, G848E, 1148insG, 4481delAG), plus two mutated alleles previously reported (499delTGTT, L847P) have been identified. In addition we detected one silent mutation (G846A), three rare intron changes (730-6)A→C, (1063-28)C→G, (1063-24)delT, and one apparent polymorphism (4368-46)G→C. Our results suggest that DHPLC provides an accurate method for the rapid identification of NF1 mutations.
This work was supported by the Italian Ministry of Health and Associazione Romana Ricerca Dermatologica (ARRD)

P0107. Mutation characterization in patients with type I Neurofibromatosis: towards a routine diagnostic test

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Neurofibromatosis type I, an autosomal dominant condition with an incidence of about 1/3000, results from mutations in the NF1 gene. The variability in clinical expression is striking, with symptoms varying from "cosmetic" to lethal. This variability is apparently not due to locus heterogeneity, since mutations in the NF1 gene are responsible for the quasi-totality of cases. Allelic heterogeneity may explain a proportion, as over 400 mutations have been reported. However, major intrafamilial variation in disease expression also occurs, making the establishment of genotype-phenotype correlations difficult. A reliable molecular diagnostic test is needed to allow earlier diagnosis, carrier detection and clinical follow-up. However, the size of the gene and the diversity of mutations currently makes testing difficult outside of a research context. We have done molecular analysis on 38 NF1 patients using a multi-step DNA and RNA-based protocol, using SSCA of selected exons, RT-PCR and PTT followed by sequencing of variants. This approach has allowed us to define mutations in nearly half (17 of 38) of our patients, with work still ongoing. The mutations defined, 11 of which have not been previously described, include 7 nonsense, 4 splice-site, 4 insertion-deletions and 2 missense mutations.
Attempts will be made to correlate clinical symptoms with specific mutations, as a standard set of clinical information has been collected.
On the basis of these and published results we now propose a routine diagnostic test which compromises acceptable cost and reasonable sensitivity, thus responding to a frequent demand of patients and physicians.

P0109. Characterization of 2p aberrations in classical Hodgkin lymphoma by means of FICTION reveals recurrent involvement of the REL and BCL11A loci

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The genetic background of classical Hodgkin lymphoma (cHL) is widely unknown. A common feature of the tumor cells in HL is the constitutive activation of the NF-kB transcription factor. In a subset of cases this might be due to the presence of the LMP1 of the EBV or mutations in the NF-kB inhibitors IkB-alpha or IkB-epsilon. Recent comparative genomic hybridization studies have shown gains in chromosome arm 2p as the most common imbalance in cHL. The minimal region of gain contained two candidate oncogenes, REL and BCL11A. Overexpression of REL due to genetic amplification might contribute to NF-kB activation. The transcriptional repressor BCL11A has been shown to be overexpressed in cHL cell lines and may play a role in B-cell transformation through the BCL6 pathway. Here, we investigated the involvement of REL and BCL11A in 44 primary cases of cHL by combined immunophenotyping and interphase cytogenetics (FICTION technique). A median 2p13 copy number above the tetraploid range was detected in 24 (55%) cases. Adjustment for centromere 2 copy number indicated gains of 2p13 in 11 of 31 cHL (35%) with 8 (26%) high-level amplifications. One case displayed selective amplification of the REL locus not affecting BCL11A, and another case showed signal patterns suggesting a breakpoint in the region spanned by the REL probe. According to these data, REL rather than BCL11A may be the target of the 2p13 alterations in cHL, although a role for BCL11A in cHL cannot be ruled out due to its frequent coamplification.

P0110. Gene mutation in the SDHB gene in sporadic pheochromocytoma induces the same functional consequences as SDHD gene mutation in hereditary paraganglioma

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The genetics of neural crest-derived tumors was recently transformed by the discovery of mutations in SDHD, SDHC and SDHB genes. The goal of this study was to better understand the functional consequences of SDHD and SDHB gene mutations.The first patient had a mediastinal pheochromocytoma and was a member of a family with inherited paraganglioma.The second patient had a malignant nonfamilial pheochromocytoma. The search for mutations in the SDHD and SDHB genes was performed by direct sequencing in germ-line and tumoral DNA. LOH was tested with several fluorescent microsatellites of 11q and 1p chromosome regions. The activity of respiratory-chain enzymes was tested by measuring the succinate cytochrome c reductase activities on the tumor homogenates. The influence of the mutations on the hypoxic pathway was tested by in situ hybridization, immunohistochemistry and real-time quantitative RT-PCR.A nonsense mutation of the SDHD gene was detected in the first patient and a missense mutation of the SDHB gene in the second. These two mutations were associated with a LOH in tumors on 11q and 1p chromosomes, respectively. Enzymatic experiments showed a complete and selective loss of complex II electron transfer activity in both the SDHD- and SDHB-inherited pheochromocytomas. Immunohistochemistry, in situ hybridization and quantitative RT-PCR revealed a high level of expression of angiogenic factors EPAS1, VEGF and its receptors in both tumors.Mutation in the SDHB gene induced a dramatic disturbance of mitochondrial and hypoxia pathways similar to those induced by SDHD gene mutation, which might be important to trigger tumorigenesis of pheochromocytomas.

P0111. Investigation of a potential role of the putative tumor suppressor gene *EXTL1* in neuroblastoma development

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Neuroblastoma, a frequent pediatric tumor of the sympathetic nervous system, is characterized by a wide variety in outcome, ranging from rapid progression of the disease associated with poor prognosis to a quite unusual and rather high rate of spontaneous tumor regression that correlates with excellent prognosis. It has been shown that molecular abnormalities often recognized in neuroblastomas, such as *MYCN*-amplification or deletion of the distal part of chromosome 1p are correlated with the outcome of the disease. Two neuroblastoma suppressor loci are thought to be located on chromosome 1p36. Recently, the *EXTL1* gene was suggested to be a candidate neuroblastoma suppressor gene because of its chromosomal localization in the 1p36.1 region between the translocation breakpoints observed in the UHG-NP and GI-ME-N neuroblastoma-derived cell lines, and its presumed tumor suppressor capacity. To evaluate this hypothesis, we performed 1p-deletion analysis and mutation screening of the *EXTL1* coding region on tumor genomic DNA originating from 25 neuroblastoma-derived cell lines and 30 neuroblastomas. Deletion of a fragment of chromosome 1p, including the *EXTL1* locus, has been observed in at least 23 of the 55 investigated neuroblastoma samples. Only one mutation (C83G; Ser28Cys) has been observed in the DNA of neuroblastoma cell line STA-NB3. This combination of deletion and mutation analysis allows us to conclude that the *EXTL1* gene does not play a leading role in neuroblastoma etiology, despite the fact that it is often deleted.

P0112. Plasma DNA Microsatellite Analysis for Bladder Cancer Follow-up may give False Positive Results.

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Purpose: Patients with non-invasive bladder cancer develop frequent recurrences, a part of which progresses into invasive disease. Intensive follow-up is therefore required.
We studied the possibility of following-up bladder cancer patients by microsatellite investigation of plasma DNA for LOH (Loss of Heterozygosity) and MSI (Microsatellite Instability).
Materials and methods: Sixteen microsatellite markers were amplified in plasma, leukocyte and, when available, tissue DNA of 40 patients and 20 healthy controls by use of fluorescent primers. The plasma was filtered prior to DNA extraction in order to exclude the presence of cells.
Results:

Classification	Tissue LOH/cases analyzed	Plasma LOH/cases analyzed
Controls	0 % (0/10)	5 % (1/20)
Patients (Ta + T1-4)	68 % (25/37)	25 % (10/40)

Only two patients displayed LOH in both tissue and plasma, however the markers involved were not the same.
The tissue MSI frequency was 0 % in the controls and 5 % in the cancer patients. As for the plasma MSI, in most cases it was enough to reduce the number of PCR cycles in order for it to disappear.
Conclusions: Plasma microsatellite analysis was found to be of little use for bladder cancer follow-up. Attention should be paid to allelic drop-out and overamplification as they may give, respectively, artifact LOH and MSI.

P0113. Cytogenetic investigation of 224 Leukaemic cases in Iran

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The investigation was carried out on bone marrow and peripheral blood samples of 224 Iranian patients presented or followed up for various types of leukaemia in years 1999 and 2000. The samples were mainly referred from three major haematology-oncology centres at Tehran. The Cell cultureing and bandings were carried out according to standard protocols. Chromosome analysis was performed following ISCN guidelines. The patients were from different leukaemic groups, the major ones being: CML, AML, ALL and MDS. There were more males than females: 133 and 91 respectively with approximately 1.5:1 ratio. In terms of sample types, 210 had BM aspiration, whreas peripheral blood was used only in 14 cases. The common typical chromosome abnormalities as well as rare and combined forms were observed. The overall chromosome abnormality rate obtained was about 50%. The breakdown figures for different categories were as follows: 73% in CML, 41% in AML, 26% in ALL, 30% in MDS and 61% in other types.
Compared to the published data, the observed rate in the present study is considered low to average. The main reason being the patients selection criteria at the initial diagnosis stage.

P0114. The role of microsatellite instability in patients with gastric cancer: A case-control study in high-frequent area.

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Introduction: Microsatellite instability (MSI) in gastrointestinal cancers is a process in developing of gastric cancer. We studied MSI in 8 MS region in gastric cancer tissue of the patients with a history of gastric cancer in their first-degree-relative and healthy family memebers.
Methods: Patients expired of gastric cancer with a positive family history of gastric cancer in the first-degree relative and their healthy first-degree relatives were included in the study. Paraffin-blocked tissues were obtained from pathology department. Biopsies from the intact gastric tissue were also taken from healthy members. DNA was extracted and reserved in 4oC. MSI was checked based on 8 MS markers including BAT25, BAT26, BAT40, D2S123, D5S346, D13S170, D17S250 and TP53.
Results: 20 patients were enrolled in the study. Low-level MSI was detected in 9 (45%) and high-level MSI in 3(15%). 8 patients (40%) was MSS. 20 healthy members were randomly selected and included in the study. Low-level MSI was detected in 4 (20%) and the other cases (85%) were MSS. Intestinal metaplasia was detected in 2 patents with low-level MSI.
Conclusion: This study showed that MSI could be one of the most important screening tests in detecting first-degree relatives of patients with gastric cancer.

P0115. Hydatidiform mole (HYDM): study of three large Indian pedigrees

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Hydatidiform mole (HYDM) (OMIM 231090) is the product of malformed human pregnancy with the incidence ranging from 1/250 to 1/1500 pregnancies depending on ethnic groups. It was classified into two different types. Complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). CHM is characterized by gross hydropic swelling of almost all of the chorionic villi with loss of intravillous vascularity and resembling bunches of grapes, usually with an absence of a fetus or fetal tissue such as blood or amniotic membrane. PHM is generally accompanied by a fetus, or shows evidence of a previous existence of a fetus by the presence of erythroblasts or fetal membrane. The gene responsible for HYDM have been mapped to chromosome 19q13.3-q13.4 (Hum. Molec. Genet. 8:667-671, 1999; Europ. J. Hum. Genet. 8: 641-644, 2000). We have studied three large Indian pedigrees with an autosomal recessive HYDM. Pedigrees consist of 65 individuals, including 18 affecteds. The Severity of the disease was quite variable among the families. The desease occurred in two or more pregnancies in two or more sisters of the same pedigree. Detailed clinical, ultrasonographic, morphological and histological studies were carried out on 14 affecteds from the three pedigrees. The affected status of remaining individuals was collected from the family records. Karyotype analysis of six affecteds representing the three pedigrees showed no chromosomal anomaly. Linkage studies with markers closely linked to HYDM will either confirm allelism to this locus or provide evidence for genetic heterogeneity.

P0116. Polymorphisms C825T in GNB3 gene and Pro/Leu at codon 10 in TGFbeta1 gene and the risk of prostate cancer

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Essential role for G proteins and TGFbeta in prostate cells signaling, growth and differentiation was recently recognized. Beta 3 subunit is functional part of most G proteins signaling cascades with its polymorphism C825T being functionally significant. TGFbeta1 level was found to be predictor for progression of prostate cancer and polymorphism in codon 10 is associated with variations in tissue TGFbeta1 level.
A group of 86 patients with histologically proven prostate cancer was compared to 200 apparently healthy controls.
C825T polymorphism distribution was 14% TT, 44% CT, 42% CC among patients and 8% TT, 46% CT and 46% CC among controls. The difference for the frequency of homozygosity TT was not significant between the groups (p=0.12, OR 1.87, 95%CI 0.84-4.1). Proline (C) and leucine (T) distribution among cancer patients was 11% CC, 55% CT, 34% TT and among controls 17% CC, 49% CT, 34% TT. The difference for the frequency of homoygosity CC was not significant between the groups (p=0.25, OR 0.64, 95%CI 0.30-1.37). The two studied polymorphisms were not found to be significantly correlated with the risk of prostate cancer in Slovenian/Caucasian population, although trend toward increased risk for TT in C825T GNB3 was noted. These findings do not exclude influence of these polymorphisms on progression and prognosis of prostate cancer, which warrants further studies.

P0117. Genetic changes of chromosomal region 10q24 in malignant lymphomas: Detection of aberrations affecting the NFKB2/LYT10 gene locus by FISH

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Various genetic changes affecting chromosomal region 10q24 have been shown to be recurrent in malignant lymphomas. Among those, the translocation t(10;14)(q24;q32) is supposed to activate the NFKB2/LYT10 gene in 10q24 via its juxtaposition nexto the IGH gene in 14q32. In addition, the NFKB2/LYT10-locus has been reported to be targeted by deletions particularly in cutaneous lymphomas.
In the present study, we established an interphase FISH assay for detecting alterations of NFKB2/LYT10-locus. As probes, differentially labelled BAC-clones from a contig of chromosomal region 10q24 were applied which cover at least 400kb on each side of the NFKB2/LYT10-locus. The diagnostic cut-off levels of the FISH probe set in interphase cells were determined in normal controls. A cell line known to carry breaks in both NFKB2 alleles served as positive control. In all five studied B-cell lymphomas with cytogenetically proven t(10;14)(q24;q32) a break within the IGH locus but not in the NFKB2 locus was detected. From 14 lymphomas with chromosomal

aberrations in 10q, only a single case displayed a signal constellation indicating a breakpoint within the NFKB2/LYT10-locus. Four cases showed signal patterns indicating deletions of the NFKB2/LYT10-locus. Similarly, a loss of the NFKB2/LYT10-locus was detected in 8/18 cutaneous T-cell lymphomas. These findings indicate that the NFKB2 gene very likely is not the sole target of the t(10;14)(q24;q32) and that deletions affecting the NFKB2/LYT10-locus are recurrent particularly in cutaneous T-cell lymphomas. Finally, our results show the established double-color FISH assay to provide a new routinely applicable tool for diagnosing recurrent breakpoints and imbalances in NFKB2/LYT10-locus.

P0118. p16INK4a, p15INK4b, p14ARF, Rb1 and ECAD Genes Aberrant Methylation in Various Cancers.

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Alterations of DNA methylation pattern have been recognized as common changes in human cancers.
We investigated the frequency of p16INK4a, p15INK4b, p14ARF, retinoblastoma (Rb1) and E-cadherin (ECAD) genes aberrant methylation in different cancers: breast cancer (60 samples), non-small lung cancer - NSLC - (35 samples), nephroblastoma (10 samples), retinoblastoma (50 samples), acute lymphoblastic leukemia - ALL - (30 samples), chronic lymphoblastic leukemia - CLL - (15 samples).
Methylation in the breast cancer samples was detected in 14% for Rb1 promoter, 27% for p16INK4a promoter, 41% for p16INK4a exon1, 40% for ECAD promoter; for nephroblastoma - 70% for Rb1 promoter, 50% for p16INK4a promoter, 50% for p16INK4a exon1, 50% for ECAD promoter; for NSLC - 12% for Rb1 promoter, 18% for p16INK4a promoter, 66% for p16INK4a exon1, 72% for ECAD promoter; for ALL - 16% for Rb1 promoter, 26% for p16INK4a promoter, 26% for p16INK4a exon1, 20% forECAD promoter; for CLL - 13% for Rb1 promoter, 26% for p16INK4a promoter, 26% for p16INK4a exon1, 6% for ECAD promoter; for retinoblastoma - 28% for Rb1 promoter, 16% for p16INK4a promoter, 57% for p16INK4a exon1, 59% for ECAD promoter. No methylation of p15INK4b and p14ARF was detected in our samples. In a number of samples we have shown joint methylation of several genes.
Studies of joint genes methylation and determination of methylation profile in tumors will allow to define a functional role of genes in carcinogenesis, as well as to develop practical approaches to the early diagnostics of cancer.

Joint methylation of p16INK4a, Rb1, ECAD genes in cancer samples

	p16 Rb1	p16 ECAD	Rb1 ECAD	p16 Rb1 ECAD
ALL	9%	9%	6%	6%
CLL	12%	6%	6%	6%
Breast cancer	14%	51%	8%	8%
NSLC	11%	53%	11%	11%
Retinoblastoma	16%	42%	18%	12%
Nephroblastoma	40%	50%	40%	40%

P0119. Frequency of the C282Y and H63D mutations of HFE gene in patients with malignant glioblastomas from Ukraine

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The discovery of the HFE gene allows us to study the molecular basis of iron overload disorders. In hereditary haemochromatosis high frequency of the C282Y and H63D mutations of HFE gene established, but their role in neoplastic transformation are still under investigation. For elucidation the association beetween HFE gene mutations and risk malignant gliomas development we investigated the frequency of the HFE mutations on DNA of 38 patients with malignant glioblastomas and 97 normal healthy subjects from Ukraine. The C282Y and H63D mutations were detected after PCR amplification of exons 2 and 4 HFE gene followed by restriction endonuclease digestion with RsaI for C282Y and BclI for H63D. Statistical data analysis was performed by Fishers exect test.
The allele frequency of the C282Y mutation in the normal population and malignant glioblastomas patients were 0.021 and 0.026, respectivelly (p=0.32). The allele frequency of the H63D mutation in the normal subjects and malignant gliomas patients were 0.17 and 0.13, respectivelly(p=0.11).
These findings did not provide evidence of association between higher level of H63D mutation and malignant glioblastomas in contrast to the date obtained by F. Martines di Montemuros et al. (2001).The obtained tendency of association malignant glioblastomas with lower frequency of H63D mutation in genotype will be check for major patients group with malignant glioblastomas.

P0120. A Complex Karyotype in A Childhood Relaps ALL-L1

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It is known that clonal chromosomal changes in childhood ALL are nonrandom. They are important markers for diagnosis, prognosis and relaps. The median survival time following relaps and clonal complex chromosomal changes are related. Our case was four years old boy. He was diagnosed with ALL-L1 a year ago. He had weakness, fever, massive lymphadenopathy (LAP) and hepatosplenomegaly. He was considered poor risk patient and treated with chemotherapy and radiotherapy because of central nervous system involvement. Relaps was occured a year later. Bone marrow sample was analysed cytogenetically after relaps. The karyotype was 46,XY,t(3;17)(q23?;p13),t(5;12)(q31;p13),inv(11)(p15q12)[11]/46,XY[8]. The patient died from ARDS after convulsions likely due to toxicity of drugs. t(5;12)(q31-33;p12-13) was reported in childhood and adult myelodysplastic syndromes. Extensive research of literature failed to demonstrate any published on t(3;17)(q23?;p13) in leukemias. Whereas the breakpoint of 17p13 had been involved in a t(11;17)(q23;p13) in ANLL. inv(11)(p15q12) has not been reportedin literature, but the breakpoint of 11p15 had been reported in inv(11)(p15q23) in ANLL and MDS and in t(7;11)(p15;p15) in ANLL and CML in blast crisis.
To our knowledge there is no reported case with such karyotypic abnormalities. Since the patient had not been referred us for cytogenetic examination at the time of diagnosis,it remains unknown whether this anomaly was present in earlier stages of the disease or occured after relaps.

P0121. The role of H-ras gene in tumorigenesis of oral squamous cell carcinoma

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The aim of the present study is to establish the relationship between mutations in exons 1 and 2 of the H-Ras gene and clinicopathological features of oral squamous cell carcinoma (OSCC). It is well known that H-Ras gene is one of a family of Ras genes, which encode p21 plasma membrane protein involved in signal transduction. Point mutations within codons 12 and 13(exon 1) or codon 61(exon 2) are frequently present in various tumours including OSCC. In our study, in order to detect these mutations, we isolated DNA from 20 paraffin embedded OSCC specimens. Using PCR technique, it was possible to analyse 10 of 20 (50%) samples, probably, because of DNA degradation, which occurred during paraffin embedding. According to the TNM staging, 5 of 10 successful amplified samples were stage III and 5 stage II. These histopathological parameters suggest that our analyzed samples, might have a higher incidence of H-Ras mutations, but to confirm this correlation, PCR products will be screened by method for detection mutations–SSCP. Also, the additional cases will be tested and their prognostic significance will be discussed.

P0122. Translocation T(1;21)(p36;q22) In A Child With Fanconi Anemia

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We report herein a 9-year old boy undergoing a cytogenetic investigation for pancytopenia. A bone marrow aspirate revealed aplastic anemia. A diagnosis of fanconi anemia was confirmed by an increased number of chromosomal breaks and rearrangements in peripheral blood lymphocytes cultured in the presence of mitomycin C. The patient had no phenotypic manifestations. Bone marrow RHG-banded karyotype showed deletion del(21)(q22). Fluorescence in situ hybridization (FISH) analysis revealed a cryptic translocation t(1;21)(p36;q22).This translocation have been reported previously in two old patients who developed acute myeloid leukemia secondary to toxic exposure (after radiation exposure in one case, and after treatment with antitopoisomerase II in the second case), but this is the first report of t(1;21)(p36;q22) in a child with fanconi anemia.

P0123. Neuroblastoma cell detection by RT-PCR for tyrosine hydroxylase mRNA

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Bone marrow metastasis often occurs in patients with neuroblastoma; therefore a sensitive assay to detect circulating neuroblastoma cells in bone marrow (BM) and peripheral blood (PB) is needed. The feasibility and clinical value of using the reverse transcriptase (RT) polymerase chain reaction (PCR) to amplify mRNA for tyrosinase hydroxylase (TH), the first enzyme of catecholamine synthesis, was evaluated to detect neroblastoma cells in patient samples.
A total of 32 patients with neuroblastoma were studied. After preparation of complementary DNA, the PCR was performed to amplify the TH gene. Amplified products were analyzed in polyacrylamide gel or by using the GeneScan Analysis on ABI PRISM 310.The specificity of the amplified products was checked by sequencing analysis and neuroblastoma cells were detected at a level of 1 per 10^5 normal PB mononuclear cells by this method.TH mRNA was in 19 of the 63 BM samples, in 6 of the 53 PB samples and in 4 of the 8 tumor samples.
RT-PCR of TH mRNA is a sensitive and specific method for detection of circulating neuroblastoma cells in BM and PB samples. The clinical significance of these very low levels of neuroblastoma cells detected by RT-PCR requires further investigation. We conclude that, by combining multiple molecular markers and independent screening techniques, we may be able to overcome tumor heterogeneity and expedite the detection of microscopic disease in the clinical management of NB. This work is supported from the Ministry of the Health in the Czech Republic (MZ 0006526 97 05)

P0124. Microsatelite Instability in Bulgarian Patients with Colorectal Cancer

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Colorectal cancer (CRC) is the most common gastro- intestinal neoplasia. Hereditary nonpolyposis colorectal cancer is the most common type of familial CRC. Germline mutations in one of DNA mismatch repair genes are known to be responsible for the HNPCC phenotype. Their mutations induce microsatelite instability (MSI). Approximately 12-17% of the unselected tumors also show MSI. Highly efficient set of five markers- D2S123, BAT26, D5S346, D18S35 and FGA have been selected for detecting the MSI.
A total of 108 patients with colorectal cancer have been included in the current study. The analysis was performed using two detection methods- denaturing polyacrylamide gels followed by silver staining and/or automated fluorescence detection (ALFExpress Biotech, Pharmacia).
We detected 16 tumours with MSI. These cases were reliably detected by the two methods. 64,3% of them were family cases. In 65% of the MSI cases the tumors were right localized, compared with 27% in the whole group patients. We found strong correlation between the right tumor localization and MSI-H (MSI-high) phenotypes.
The histopathological evaluation showed the presence of mucin production in 64,3% of the MSI cases, compared with 15,9% in the microsatelite stable tumors. Most of the cases had not cancer cell metastasis in the regional lymph nodes (71,4%). All the right localized tumors were included in this group. No long distance metastases in MSI positive tumors were found. These data were corelated with the better prognosis of the MSI positive colorectal cancers especially the tumors with right colon origin.

P0125. PCR detection and typing of HPV in Bulgarian patients with invasive cervical cancer

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Human Papillomavirus (HPV) infection is considered to be an important risk factor for cervical cancer development. The polymerase chain reaction (PCR) method enables detection and typing of large number of HPV types and is widely used in HPV diagnostics. Here we present results from PCR HPV typing in cervical cancer patients obtained for the first time in Bulgaria.
HPV diagnostics was carried out on DNA samples obtained from 70 paraffin-embedded biopsies from Bulgarian women diagnosed with invasive cervical cancers during the last 2 years. The samples were initially tested with MY09/MY11 consensus primers located within the L1 region of HPV genome, which covers a broad spectrum of HPV types. Gamma globin gene amplification was carried out as an internal positive control. The positive samples were subsequently tested with specific primers for the most common HPV types (6, 11, 16, 18, 31 and 33).
We detected HPV in 45 cases (64.28%). Mixed infection was observed in more than 40% of the positive cases. HPV 16 was detected in 25 cases (31.6%), HPV 18 in 19 (24.1%), HPV 6 in 15 (19.0%), HPV 11 in 9 (11.4%), HPV 31 in 7 (8.9%) and HPV 33 in 4 cases(5.0%).
Our initial results indicate the highest prevalence of HPV types 16 and 18. This confirms the association of high-risk types HPV16 and 18 with invasive cervical cancers.

P0126. Amplification of the androgen receptor gene in primary prostate cancer due to X chromosome aneuploidy

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In the majority of patients with prostate cancers (PC), the growth of the tumor is androgen-dependent. In advanced PC, when radical treatment of prostate cancer is not curative, androgen deprivation therapy has proved to be an effective palliative therapy. In this study we analysed isolated nuclei from 31 prostatectomy specimens from PC patients without preoperative therapy for amplification of the androgen receptor (AR) gene by fluorescence in situ hybridization (FISH). For this purpose, an AR gene probe (Vysis) was used in combination with an X centromere control probe. In 11 out of 31 PC, additional X chromosomes (disomy to tetrasomy) with the corresponding AR gene could be detected in more than 20% of the analysed nuclei. In two of these patients, over 40% of the nuclei of the PC tissue showed two or more fluorescence signals. Control analyses with centromere probes of chromosomes X, Y and 18 confirmed these high level of X aneuploidies. Both patients have advanced stage PC (pT4,pN1 and pT3b,pN0). FISH analysis on normal prostate tissue detected in both patients in less than 10% of the nuclei additional X chromosomes. Furthermore, the analysis of 11 normal prostate tissues showed that an average of 8% of the nuclei had two or more signals for the AR and X centromere probe.

We conclude that additional AR gene copies are present in a subgroup of primary PC prior to anti-androgen therapy. This may be an important factor for initial anti-androgen resistance.
This study was founded by Dr. Mildred Scheel Stiftung.

P0127. Microsatellite instability in HNPCC patients

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Microsatellite instability (MSI) is characteristic feature of colorectal cancer with loss of mismatch repair (MMR) in tumor cells. MSI is found in tumors of patients with HNPCC and in 15-20% of sporadic colorectal tumors. In connection with mutation analysis of MSH2 and MLH1 genes in patients with HNPCC, we studied MSI in 47 unrelated patients with colorectal cancer. Of these, 8 patients fulfilled Amsterdam criteria (AMS+), 27 patients were familial (AMS-) and 12 were sporadic cases (Spor). Two mononucleotide (BATRII, BAT26) and five dinucleotide (D2S123, D3S1029, D5S346, D17S250, D18S58) loci were analysed. Initially, MSI was determined in denaturing polyacrylamide gel stained with ethidium bromide, then fragmentation analysis with fluorescent primers on ABI Prism 310 Genetic Analyzer was introduced and both methods were compared. Tumors were classified as MSI-H (high degree of MSI, 2 or more loci with MSI), MSI-L (low degree of MSI, 1 unstable locus), and MSS (stable, no MSI detected). Results of MSI analysis in tumors are shown in table. Number of patients with germ-line mutation in MSH2 or MLH1 gene is shown in parentheses. In patients with MSI-H tumor and negative result of mutation detection, analysis of methylation status of MLH1 gene promoter is in progress. This work was supported by Grant Agency of Charles University (Grant No. 17/2001).

Group of patients	MSI-H tumors	MSI-L tumors	MSS tumors	Total
AMS+	5 (5)	1 (0)	2 (0)	8 (5)
AMS-	5 (2)	1 (0)	21 (1)	27 (3)
Spor	2 (0)	0	10 (2)	12 (2)
All	12 (7)	2 (0)	33 (3)	47 (10)

P0128. Differential gene expression in human prostate cancer.

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Prostate cancer is the most frequently diagnosed solid tumor in men, and the second leading cause of cancer death in males from western countries. One of the key issues in prostate cancer research is to develop molecular markers that can effectively detect and distinguish the progression and malignancy of prostate tumors. In order to analyze differential gene expression of putative tumor markers labeled cDNA probes were generated from capsule-invasive prostate tumor (stage pT3a) and normal prostate tissue. The cDNA probes were hybridized with an Atlas Select Human Tumor cDNA Expression Array (Clontech) with immobilized cDNAs of differentially expressed genes from five different human tumors, e.g. bladder, breast, liver, lung and prostate carcinoma. In total, 46 known and unknown genes were identified to be up-or down-regulated in prostate carcinoma. The known genes showing a differential expression pattern in prostate tumor samples included transcription factors, protooncogenes and other proteins, e.g. Krox 24, c-jun, spermidyne acetyltransferase, ribosomal proteins, clusterin and prostate secretory protein 94. In addition, by using both Northern blot analyses on whole tumor RNA and real time RT-PCR on RNA from tumor cryosections enhanced expression of seven unknown genes was verified in prostate tumors as compared to normal prostate tissue. To further circumvent the problem of tissue heterogeneity, RNA from microdissected prostate tumor tissue samples was isolated and analyzed for differential gene expression. The identification of these tumor-specific expression patterns could lead to the establishment of genetic fingerprints of prostate tumors as a versatile tool for diagnostic and prognostic purposes.

P0129. Genetic alterations in the SDH genes lead to oncogenesis of paragangliomas

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Paragangliomas of the head and neck region are usually benign tumors developing from chemoreceptors of paraganglionic origin in the majority of patients. These receptors play an important role in sensing and regulation of the blood CO2-level. Genetic alterations in the mitochondrial enzyme complex II (SDH), which is involved in respiratory chain and citric acid cycle reactions, have been shown to lead to sporadic as well as familiar cases of these tumors. Therefore we analyzed our collective containing sporadic cases of patients with paragangliomas for genetic changes in the SDH-genes SDHD, SDHC and SDHB. We detected several new DNA mutations in samples derived from tumor patients. Furthermore we demonstrated loss of heterozygosity (LOH) usually connected with oncogenesis of various tumors. Elucidation of the genetic regions involved in tumor development is a basis for understanding their contribution to normal and pathogenic cell physiology.

P0130. A fixed cascade of genomic changes in a murine tumor progression model for pancreatic adenocarcinoma

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P53+/- knockout mice overexpressing TGFa in a pancreas specific manner develop adenocarcinoma of the pancreatic ducts. This established animal model reflects human pancreatic cancer disease. To investigate secondary genomic changes, more than 40 tumors were analyzed by CGH. In about 40% of the tumors gain of proximal chromosome 11 and loss of its distal part including p53 was detected. Due to this loss no wildtype p53-allel is left.
In addition to this recurrent aberration pattern further cancer progression follows two alternate routes: Overrepresentation of chromosome 15 including the Myc locus (A), or more rarely, loss of the distal part of chromosome 14 including the Rb-locus (B). It seems that these aberrations occur exclusively because in none of the aforementioned tumors a combination of these events was detected. On the average mice bearing tumors of subtype B develop tumors about 75 days earlier than mice with tumors of subtype A. These data indicate that there are at least two different pathways in pancreatic tumor formation: Proliferation through Rb-deletion or c-myc overexpression and activation.
To analyze the extension of the amplified regions of chromosome 11 and 15 cell lines were investigated by FISH and real-time PCR. On proximal chromosome 11 the amplification-unit extends about 18 cM. Together with Egfr, the c-Rel gene gets amplified which provides antiapoptotic activity. In contrast, the Myc amplification-unit of chromosome 15 is much smaller.
In the meanwhile a second series of pancreatic adenocarcinoma with TGFa / p19ARF+/- was analyzed by CGH. These tumors show less chromosomal aberrations.

P0131. Breakpoint Analysis of a Novel Recurrent Chromosomal Translocation (14;20)(q32;q12) in a Human Multiple Myeloma Cell Line.

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Recurrent chromosomal translocations are regularly involved in hematological malignancies. Translocations involving the Immunoglobulin Heavy chain (IgH) region at chromosome 14q32 are a hallmark in human B cell malignancies including multiple myeloma (MM). Using Fluorescent In Situ Hybridization (FISH) we already demonstrated the ubiquitous presence of translocations in this region in MM cell lines, with a diverse array of translocation partners. Recently we have found a high percentage (92%) of der14t(14;?)(q32;?) in fresh MM samples as well.

Apart from the four major 14q32 translocations (involving 4p16, 6p25, 11q13 and 16q23), we reported another recurrent translocation, i.e. t(14;20)(q32;q12). Here, we describe the cloning and characterization of the breakpoint of this UM3 cell line.
Using FiberFISH techniques the breakpoint was detected in the switch gamma-1 region of chromosome14. Next, a genomic phage library of the UM3 cell line was screened with a gamma-1 probe resulting in a clone that spans the breakpoint. Sequence analysis pinpointed the breakpoint of chromosome 14 in the mu enhancer, adjacent to the recombined switch mu/switch gamma-1 genes. Screening a P1 library picked up a larger genomic fragment covering the breakpoint at chromosome 20. Screening of a UM3 derived cDNA library with this P1 clone resulted in a cDNA clone of 3.5kb. The first known gene located downstream of the breakpoint at 20q12 is the oncoprotein MAF-B, pointing to MAF-B as one of the candidate target genes involved in t(14;20) in multiple myeloma. Functional studies to such an effect are underway.

P0132. Increased noise as an effect of haploinsufficiency of the tumor suppressor gene Neurofibromatosis type 1 in vitro

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In human diseases related to tumor suppressor genes, it is suggested that only the complete loss of the protein results in specific symptoms such as tumor formation, whereas simple reduction of protein quantity to 50%, called haploinsufficiency, essentially does not affect cellular behavior. Using a model of gene expression it was presumed that haploinsufficiency is related to an increased noise in gene expression also in vivo [Cook, D. L., Gerber, A. N. & Tapscott, S. J. (1998) Proc. Natl. Acad. Sci. USA 95, 15641-15646]. Here, we demonstrate that haploinsufficiency of the tumor suppressor gene Neurofibromatosis type 1 (NF1) results in an increased variation of dendrite formation in cultured NF1 melanocytes. These morphological differences between NF1 and control melanocytes can be described by a mathematical model where the cell is considered a self-organized automaton.
The model describes the adjustment of the cells to a set point and includes a noise term which allows for stochastic processes. It describes the experimental data of control and NF1 melanocytes. In the cells haploinsufficient for NF1 we found an altered signal-to-noise ratio detectable as increased variation in dendrite formation in two out of three investigated morphological parameters. We also suggest that in vivo NF1 haploinsufficiency results in an increased noise in a cellular regulation and that this effect of haploinsufficiency might be found also in other tumor suppressors.

P0133. RB1 structural and functional pathology, methylation pattern of p16, p15, p14 and ECAD genes in retinoblastoma patients.

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The aim of our investigation was to research molecular anomalies causing retinoblastoma. Retinoblastoma is an embryonic malignant tumor of retina with an incidence of 1:13000 which is found together with structural abnormalities in RB1 tumor-suppressor gene. Some cases are obviously caused by methylation of RB1 gene promoter region. SSCP and heteroduplex analysis were used for mutation screening, microsatellite analysis (intron 2, 20, D13S262, D13S284) - for loss of heterozygosity (LOH), methylation pattern analysis of RB1 promoter region - for functional mutation.
We have studied 60 families with different forms of retinoblastoma. Analysis of PCR products mobility by using of SSCP and heteroduplex analyses revealed 47 mutations in different RB1 gene exons and introns. All familial and sporadic bilateral cases of retinoblastoma had germinal mutations. Complete deletion of RB1 was revealed in two sporadic cases. Loss of heterozygosity of at least one of intragenic markers was found in 71% of analyzed tumors. Methylation pattern anomalies of the RB1 gene promoter region were found in 27% retinoblastomas.
Having not found any abnormalities in same tumors we undertook a study of methylation status of p16 gene (INK4a) promoter region as it functions as an upstream regulator of pRB activity and others important tumor-suppressor gene: p15 (INK4b), p14 (ARF) and ECAD in retinoblastoma tumors. We found abnormal methylation p16 promoter region - 11% cases, in exon 1 of p16 - 46% tumors and methylation of ECAD promoter - in 59% retinoblastomas. These data confirm necessity of methylation profile studies in different tumor types.

P0134. Telomerase activity as a predictive marker for in vitro chemo-reponsiveness of bilharzial bladder cancer

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The DNA component of telomeres is synthesized by a specialized reverse transcriptase enzyme,the telomerase.More than 90% of human cancers are telomerase positive where most normal tissues or benign tumors contained low or undetectable levels.We wanted to know if there would be any correlation between the in vitro sensitivity of bilharzial bladder cancer to different chemotherapeutic agents and the telomerase activity.The present study included 33 samples taken from bladder cancer patients treated at the National Cancer Institute,Cairo University.Three anticancer drugs (gemcitabine,taxotere,and navelbine) were applied as single agents or in combination.Telomerase activity was positive in 28 out of the 33(85%)cases studied.The drug gemcitabine was tested on all 33 samples included in the study,while taxotere and navelbine were tested on 26 and 28 samples respectively.Simultaneous application of the 3 drugs was tested on 7 samples. No sensitivity could be detected to any of the 33 samples tested for gemcitabine (0%).Among the 26 samples tested for taxotere,3 samples showed in vitro sensitivity (9%).Navelbine has demonstrated in vitro activity in 1/27 (9%)samples.Among 7 samples tested for simultaneous application of the 3 drugs,the activity of the combination was demonstrated in one sample(14%). There was highly significant correlation between the telomerase activity and the in vitro sensitivity to both the drugs taxotere and navelbine (p.00016 and .0005 for taxotere and .012 and .0421 for navelbine).Thus it may be concluded that telomerase activity may be used as a predictive marker for responsiveness to chemotherapy in bilharzial bladder cancer patien ts.However,clinical correlations are clearly needed before reaching such a conclusion.

P0135. Conversion of ALL-L2 with a double 12;21 translocation to JMML with a 4;11 translocation: A cytogenetic, morphological and immunophenotypic study.

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Abstract
The phenotypic conversion of acute lymphocytic leukemia (ALL) into juvenile myelomonocytic leukemia (JMML) is a rare event, especially in the pediatric population. We describe a comprehensive cytogenetic and flowcytometric study performed from bone marrow and peripheral cells from such a child enabling us to determine the origin of his JMML. A four-year-old boy diagnosed with double t(12;21), CALLA+, pre-B ALL was treated as-per BFM protocols on the low risk arm. A routine cytogenetic analysis performed at 17 months into treatment failed to detect t(12;21) in bone marrow (BM) cells. However, a novel translocation, namely, t(4;11), involving the MLL gene at 11q23 was detected in monocytes, mature granulocytes and in immature myeloid/monocytic cells. No cytogenetic abnormalities were found either in EBV-transformed B or in PHA-stimulated T lymphoid cells. Flowcytometric analysis demonstrated an asynchronous expression of the antigenic determinants in populations of granulocyte and monocytoid cells: Low levels of CD14, an unusually high level of CD15, and no CD13 or HLA-DR antigens were observed in 60% of monocytes, while 74% of myeloid cells expressed no CD13. Debate exists as to whether JMML is a disorder of the pluripotent hemopoietic stem cells or of the committed myeloid/erythroid/ megakaryocytic (GEMM) stem cell. Our results indicate that the transformation from B-cell ALL to JMML in this case, occurred, most probably in the GEMM stem cells without involving the lymphoid cell line. The role of the initial double t(12;21) aberration in the evolution of the disease is not clear.

P0136. Leucosis virus influence on animals cells genome

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Many items of human and animals leucosis pathogenesis are not clear. We studied leucosis virus influence on animals' cells genome and change of exchange processes in an organism in case of long term infection. As a model we used Bovine Leukemia virus (BLV) and cattle of black-mottle species (in fifties experimental and check animals). Peculiarity of cattle pathogenic properties is its tropism to lymphocytes. After have studied locus BM-315 of chromosome #5 cattle's lymphocytes of infected cattle within 4 months we had defined 1 allele sized 152 pairs of nucleotides. In 4 months two alleles sized 152 and 166 pairs of nucleotides were found in the same animals. One animal in the studied locus on first analyses were found two alleles sized 160 and 156 pairs of nucleotides. In 4 months in the same animal were found 2 alleles sized 158 and 152 pairs of nucleotides. In locus MAF-50 of cattle's cells chromosome #4 was also registered change of allele's number and sizes in comparison with check animals. Thus was found the change in parts of animals' cells chromosomes #4 and #5 in BLV infected animals. Comparative RAPD-PCR analyses revealed individual changes on strips quantity in RAPD-spectrum of experimental animals in ontogenesis in comparison with check animals after a time. This testifies to changes in genome of studied cells. RAPD-PCR analyses is more informative for small sampling of animals evaluation. It is also preferable for comparing with analyses of single high-polymer locuses.

P0137. Detection of coding microsatellite instability by MALDI-TOF mass spectrometry.

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Insertion as well as deletion of repetitive units in microsatellites arise as a consequence of DNA polymerase slippage during DNA replication. The DNA mismatch repair (MMR) system eliminates the initial insertion-deletion loops to reduce the error rates in DNA replication. Defects in the MMR system result in an increased loss or gain of repeat units in microsatellites, commonly known as microsatellite instability (MSI). These processes play an important role in cancer development and therefore serve as markers for tumorigenesis. Due to this reason, efficient methodical approaches for the reliable detection of MSI with prospects for high throughput screening are needed. Matrix assisted laser desorption ionization - time of flight - mass spectrometry (MALDI-TOF-MS) possesses these general features as shown for genotyping of single nucleotide polymorphisms (SNPs). Here, we present a MALDI-TOF-MS based genotyping approach for MSI affecting mono nucleotide repeats in somatic tissues important in hereditary nonpolyposis colorectal cancer (HNPCC). Following PCR amplification of genomic regions including the microsatellites and primer extension reactions over the microsatellites to produce informative molecular masses, MSI was detected by MALDI-TOF-MS. The analysis of peak integral ratios in a single spectrum of the peaks representing insertions or deletions in comparison to the full length microsatellites lead to a relative quantification of MSI. MALDI-TOF-MS based genotyping results were confirmed completely by conventional DNA sequencing and electrophoresis. Due to its accuracy, short runtimes and low costs, this procedure possesses the potential for high throughput screening of MSI to replace gel based methods.

P0138. Chromosome mechanisms involved in the inactivation of hSNF5/INI1 leading to rhabdoid tumors.

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Rhabdoid tumors are highly malignant pediatric cancers. Observations of biallelic alterations or deletions of hSNF5/INI1 in these tumors as well as in murine models provide evidence of a tumor suppressor gene acting in this pathology. A precedent work demonstrated that the major mechanisms associated to the inactivation of hSNF5/INI1 are mitotic recombinations or putative non disjunction/duplication leading to partial or total isodisomy.
In an attempt to further characterize the main chromosomal mechanisms involved in the hSNF5/INI1 inactivation in rhabdoid tumors, we report here the molecular cytogenetic data obtained with 18 rhabdoid cell lines harboring hSNF5/INI1 mutation or deletion using 10 different markers located all along the chromosome 22q11.2-q12 region. The FISH results show that several deletions may occured in the cases carrying chromosome 22 abnormalities. The translocations including the hSNF5/INI1 consensus region are always associated with an homozygous deletion of variable size. By contrast, no deletion could be detected in any of the cases exhibiting an apparently normal karyotype.
Besides common mechanisms of mitotic recombination or non disjunction/ duplication occuring in 60% of either retinoblastoma or rhabdoid tumors, other specific chromosome mechanisms seem to be involved in each category of tumors for inactivating the respective tumor suppressor genes. Translocations associated with homozygous deletions and monosomy cases associated with mutations are mainly described in the rhabdoid tumors. Inactivation by two different mutations is mainly found in retinoblastoma. These differences may be related to the presence of low copy repeat families in the proximal 22q region leading to an increased chromosome instability.

P0139. Application of FISH and microsatellite markers for monitoring chimerism in Bulgarian patients after bone marrow transplantation

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Development of effective molecular-diagnostic methods for monitoring chimerism is an important tool for determining the risk of relapse in patients after allogenic bone marrow transplantation. Evaluation of the chimerism status in these patients provides substantial information about the replacement of host cells with donor cells during the posttransplantation period at the level of extremely small number of cells. Two male patients were studied by FISH with alternatively labeled X and Y probes after transplantation from sister and mother donors respectively. In the first case the percentage of donor cells increased in cultured lymphocytes from periferal blood from 6 to 70% in about 10 days period starting one month after the transplantation and by now the patient has achieved full remission. The second patient showed 97% chimerism on a bone marrow smear at the 51st day of transplantation and is still under observation. The three male-to-male donor-recipient pairs were studied by genotyping of ACPP, D3S1282, D3S1509, SST, RHO, D3S1212, SLC, SERT and DAT1 microsattelite loci by PCR. In the case of brother donation SERT proved to be informative and showed chimerism 3 months after the transplantation in blood sample followed by recurrence of the illness. In the case of unrelated donor ACPP was found to be informative but unfortunately the patient died before the first monitoring. In the third case (father donor) none of the markers was informative. Polymorphic microsattelite markers and FISH can be used as powerfull tools in identification of donor-recipient differences.

P0140. FISH analysis of 11q13 gene amplifications on tissue microchips of transitional cell carcinomas from Bulgarian patients

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Gene amplification results in an increased dosage of the affected gene. It represents one of the major molecular pathways through which the oncogenes are activated during tumorogenesis. The most frequently amplified proto-oncogenes in human tumors mainly belong to one of the three erbB, ras and myc families, or to the 11q13 locus. Candidate oncogenes located at 11q13 are CCND1 (PRAD1, bcl-1), EMS1, FGF3 (Int-2) and FGF4 (hst1, hstf1). We report results from a screening for gains and amplifications of CCND1, FGF3/FGF4,

FGF3 and EMS1 by fluorescence in situ hybridization (FISH) using tissue microarray (TMA) containing urinary tract tumor samples from 207 Bulgarian patientsAll 4 genes were successfully analysed in 106 tumors. Three of these tumors (2,83%) had the 4 genes amplified, three tumors (2,83%) had amplifications of CCND1, FGF3/FGF4 and FGF3 together, two of which had gain for EMS1 and one was normal for EMS1. No tumor had amplification of a single gene. 8 of the tumors had gain for all 4 genes (7,5%), 3 tumors (2,83%) had gain only for CCND1, 2 tumors (1,9%) had gain only for CCND1 and FGF4 and 1 tumor had gain for all 3 genes without CCND1. Three of the patients had Balkan Endemic Nephropathy (BEN). Interestingly, two of the three BEN tumors studied (stage pTa and pT3 respectively) had genetic gain at 11q13, the third did not react. The BEN associated pTa tumor was the only pTa tumor with 11q13 copy number change of all studied tumors.

P0141. Association of the G289S polymorphism in the HSD17B3 Gene with Prostate Cancer Risk in Italian Men

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Prostate cancer is a significant public health problem. Substantial data support an important role for androgens in the etiology of this disease. The human HSD17B3 gene encodes the testicular (or type III) 17ß-hydroxysteroid dehydrogenase enzyme which is involved in testosterone biosynthesis in men. We have investigated the G289S (glycine at codon 289 replaced by serine) polymorphism at the HSD17B3 locus as a potential candidate SNP (single nucleotide polymorphism) for prostate cancer risk in constitutional ("germline") DNA from 103 Italian prostate cancer cases and 109 Italian "centenarian" controls to assess the role of this SNP in susceptibility to prostate cancer. The G289S polymorphism appears to confer a statistically significant increased risk for prostate cancer (OR= 2.5; p= 0.04) in this pilot study.
Our preliminary data are consistent with an important role of the G289S SNP in prostate cancer susceptibility. Thus, the HSD17B3 gene may be an important candidate gene for prostate cancer risk.

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The G289S polymorphism at the HSD17B3 Locus and Prostate Cancer

Genotype	Controls	Cases	
GG(wt)	101	86	
GS+SS	8 (8+0)	17(15+2)	OR=2.5
			p=0.04

The heterozygote (GS) and homozygote (SS) genotypes were combined in the analysis. GG (wt) is the normal genotype. OR is the odds ratio and p is the p-value

P0142. A Single DNA chemical modification by cisplatin may cause long-range changes in protein binding to DNA. Results of computer modeling.

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Anticancer drug cisplatin covalently binds to DNA *in vivo*. The sites of DNA platination are recognized by several nuclear proteins. In particular, histone H1 and some of HMG proteins bind to platinated DNA with binding constants hundred times greater in comparison with unmodified DNA. Using computer modeling, we investigated binding of proteins to DNA treated by cisplatin. Binding of proteins to DNA is characterized by contact cooperativity as well as long-range interaction. Each platinated site is characterized by 100 times higher statistical weight for protein binding in comparison with non-modified ones. As a result the map of binding, i.e. the probability of each DNA base pair to be bound to a protein was calculated. It was found that chemical modification of one or several DNA base pairs by cisplatin strongly changes the character of protein binding to DNA. This effect is strongly dependent on the position of modified site. It was shown that a single chemical modification of DNA gives rise to a dramatic rearrangement in protein positions. If one protein covers 15 base pairs then the area of rearrangement involves up to 200 base pairs. Cisplatin changes the region(s) of preferable binding of proteins. In the case of chromatin, platination might cause primary nucleosome repositioning and strong secondary stabilization of their locations.
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P0143. Mixed Polyposis Syndrome in patients referred to genetic counselling clinic as Familial Adenomatous Polyposis patients.

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The polyposis syndromes are a heterogeneous group of disease characterized by multiple lesions in the intestinal tract. The classification of polyposis syndromes is based on clinical observation of affected pedigree, on hystopathological diagnosis, on extracolonic manifestations. The better characterized autosomal dominant syndromes predisposing to colorectal cancer are associated to APC gene known to cause familial adenomatous polyposis (FAP) and to mismatch repair genes hMLH1 and hMSH2 associated with hereditary nonpolyposis colorectal cancer (HNPCC). Peutz Jegher syndrome (PJS) and juvenile polyposis (JP) are characterized by amartomatous or hyperplastic polyps readily distinguishable from adenomatous polyps of FAP. Recently the mixed hereditary polyposis syndrome (HMPS) has been described as a new rare syndrome as only two small families and a large kindred were reported. Genetic linkage suggested that the HMPS locus lies on the proximal part of the long arm of chromosome 6. We report our experience in genetic counselling with 53 patients referred as FAP patients. In 32 out of 46 patients with adenomatous polyps APC gene mutation was detected. 7 presented an hystological feature of mixed adenomatous and hyperplastic polyps. No APC gene mutation or microsatellite instability (MSI) were detected. Three presented familiarity, four were apparently sporadic. Mean age of polyps detection was 40.5 (13-57). Increasing cases of mixed polyposis syndrome are seen in genetic counselling clinic but many questions raises about its classification and about surveillance of patients and at risk individuals. The syndrome is rare but an effort should be done, through collaborative studies, to identify the responsible

P0144. Germline mutations in the ccm1 gene, encoding krit1, in patients with cerebral cavernous malformations.

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Cerebral cavernous malformations (CCM) are congenital vascular anomalies of the brain, constituting approximately 10 to 20% of cerebral vascular lesions and with a frequency of 0.5% in the general population. The most common symptoms in affected patients are seizures, focal neurological deficits, migraine and/or intracranial haemorrhage, although some patients are asymptomatic.
Both sporadic and familial forms have been identified. Familial forms exhibit autosomal dominant inheritance with variable expression. In familial patients multiple lesions occur and the frequency of haemorrhages is higher. About 50% of familial forms are linked to mutations of the CCM1 gene (Chr 7q21-22) encoding Krit1 protein (736 aa). To date 27 mutations have been described all leading to non-sense stop codons and to a truncated protein.
We analysed 18 unrelated patients/families (11 sporadic cases, 2 familial cases and 5 unclassified cases) by the SSCP technique and sequencing analysis.
In the 2 familial cases, we found 2 different mutations leading to a truncated protein:
- 1302delGAAT, previously described as giving rise to a protein of 435 aa

- IVS8 -13 C→G, a new intronic substitution leading to a protein of 385 aa.
Moreover, a GTA→GTG (V660V) polymorphism was also observed in control subjects.
Using 6 extragenic polymorphic markers, we are evaluating the loss of heterozigosity in tumour tissue from CCM patients.
The aim is to identify CCM1 gene mutations and to determine the incidence of familial cases in the Italian population. Moreover, we propose to ascertain whether the "Knudson's double-loss mechanism" is involved in CCM disease.

P0145. The 825C allele of the gene GNB3 encoding the G-protein -3 subunit is associated with an increased risk for developing colorectal cancer

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Colorectal cancer is one of the major malign disease in all Western countries. Little is known about the pathogenesis of this disease. Like many other disorders, the development of colorectal cancer is multifactorial with a certain genetic contribution. We screened patients from Tirol with colorectal cancer for the C825T polymorphism in the gene encoding the G-protein beta-3 subunit. The frequency of the genotypes was 3% TT, 35% TC and 63% CC (n=157) which is significantly ($p<0.0013$) different from a local control population (randomly recruited healthy blood donors). The frequency of genotypes in the control population was 11% TT, 43% TC and 46% CC (n=188). Carriers of the 825C allele have a 1.89 fold increased risk (95% CI, 1.32-2.72) to develop colorectal cancer compared to carriers of the 825T allele. We observed also a gene-dose effect since homozygotes for 825C-allele have a higher risk to develop colorectal cancer than heterozygotes, both compared to homozygotes with the T-allele. CC versus TT gives an odds ratio of 4.83 (95% CI, 1.67-17.01) and CC versus CT gives an odds ratio of 1.70 (95% CI, 1.06-2.72). The protein of the GNB3 gene is part of a G-protein heterotrimer involved in signal transduction. The messenger RNA with the 825T allele is spliced differently but the resulting protein is still active. There is evidence that the 825T allele is associated with enhanced G-protein reactivity and hypertension, obesity and lately, that it is a genetic marker for enhanced T cell response.

P0146. Chromosome Insitability and Sister Chromatid Exchange Studies in Patients with Esophageal Carcinoma

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Cytogenetic studies have been carried out using convansional technique in peripheral blood lymphocytes in patiens with cancers. Sister chromatid exchanges (SCE) are resiprocal exchanges between sister chromatids. It has been reported that the ferquency of SCE in peripheral blood lymphocytes is significantly higher in patiens with variety type cancer than in normal individuals. This study assesed the frequencies of SCE and chromosome abnormality in peripheral lymphocytes of 28 patients with esophageal carcinoma and 20 controls. Peripheral lymphocyt cells were cultured with conventional culture methods. The blood samples were obtained from the patiens after histopatologic confirmation of the malignancy but before the initiation of chemotherapy or radiotherapy. The frequncy of aberrant metaphases appear to be significant in patients with esophageal carcinoma. The mean SCE ferquencies were 10.46 ± 0.48 and 6.82 ± 0.38 per methaphase in patients and controls, respectively. The increase of SCE frequency in cancer patients was stastically significant ($p< 0.001$), but no seen in controls. Our results suggest that patients with esophageal carcinoma show a degree of chromosomal insitability that might be related to a predisposition to neoplasia.

P0147. Cytogenetic and molecular genetic characterization of patients with neuroblastoma in Yugoslavia

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Diagnostic of neuroblastoma (NB) is complex process involving multiple analyses from different samples (blood, urine, bone marrow, tumor tissue). Determination of genetic parameters involved in NB is one of the most significant analysis with great prognostic value.
In past ten years, in Laboratory of Medical Genetics, Mother and Child Health Institute "Dr. Vukan Cupic", Belgrade, 80 patients with NB were analysed. Samples from bone marrow were cytogenetically analysed in 78 cases using standard preparation of chromosomes and G banding. In 3 patients beside cytogenetic we used FISH technique for detection of LOH for 1p36 and in one patient we combined these two techniques with PCR to detect deletion of 1p36. Two patients were analysed using just one molecular technique, FISH for 1p36 deletion in first case and PCR for 1p36 deletion in second case.
Results were following: 44 of 78 (56,4%) cytogenetically examined patients had normal karyotype while 34 (43,2%) had aberrant karyotype. 1p36 deletion was detected with FISH technique in one case while other analysed patients had no 1p 36 deletion. Cytogenetic findings in two of these trhee patient were normal but the one with deletion had also hiperdiplod clone in bone marrow. Analysis with PCR in two patients for 1p36 region showed intact chromosome 1.

P0148. Somatic mitochondrial mutation in early gastric cancer.

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Mitochondrial abnormalities have been observed in many human cancers,including changes in structure, number,respiratory enzyme components and transport systems.Mitochondrial DNA (mtDNA) can be modified by many carcinogens because its repair is less efficient compared with nuclear DNA.The mtDNA non-coding region, which contain hypervariable regions HV1 and HV2, origin of replication, the D-loop region and both origins of transcriptions,exibits a high degree of sequence polymorphisms. In this study, we examined in some gastric adenocarcinomas the mitochondrial D-loop region.The 15 primary gastric cancers were obtained from gastrectomies.MtDNA was extracted from paraffin-embedded tissues.A 445-bp portion of the human mitochondrial D-loop (bases 75 to 520) was amplified with four sets of overlapping PCR primers.For each tumor and normal fraction was performed the PCR-SSCP gel analysis.In four cancers, similar but altered bands distinctly different from the patterns obtained from adjacent normal tissue were observed. These altered bands were cloned and sequenced to reveal identical 50-bp deletions that involved flanking 9-bp direct repeats.This deletion eliminates a functional region.Antimitochondrial immunoreactivity was revealed in the supranuclear portion of adenocarcinoma cells.The deletion was not observed in normal mucosa.Mitochondrial mutations in human solid tumors have been reported in isolated case reports.One recent study sequenced a portion of the mitochondrial D-loop in colorectal cancers and failed to find mutations.Instead, in our study the 50 bp deletion was found in four out of 15 adenocarcinomas. These findings document the presence of somatic mitochondrial alterations in gastric cancer, which may reflect the environmental and genetic influence operative during tumor progression.

P0149. Blast Crisis in Philadelphia Negative CML: Clinical Findings and Molecular Cytogenetic Characterization of a 47,XX,+i(11)(q10) Cell Line.

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The presence of an abnormal karyotype is well known in Philadelphia negative CML. Cases without detectable cytogenetic aberrations are rare. The correlation between cytogenetic, morphologic, and clinical data is widely unclear. We report on a female patient who was first diagnosed with CML at an age of 69 years. At this time cytogenetic

evaluation revealed a normal karyotype and no BCR/ABL transcripts could be detected using polymerase chain reaction (PCR). Litalir therapy was performed and after a chronic phase of three years she developed a CML blast crisis. The blasts where characterized as FAB M4 and cytogenetic evaluation was performed. A clone with a 47,XX,+i(11)(q10) karyotype resulting in a partial tetrasomy for 11q could be found. Cytogenetic data was confirmed by molecular cytogenetic analysis. Palliative Aloxan therapy was applied. The patient died in the 9th month of the blast crisis.
To our knowledge the presence of an isochromosome 11q in a CML blast crisis has never been reported before. The possibility that the 47,XX,+i(11)(q10) clone developed from the CML cell line or the occurrence of a secondary leukaemia will be discussed.

P0150. Rapid quantitative monitoring of mixed chimerism using amplification a highly discriminative PCR-STR system after bone marrow transplant

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Monitoring the engraftment of donor cells after allogeneic blood stem cell transplantation (BSCT) is an important way for the early diagnosis of graft failure or relapse of disease. Several techniques have been reported for this purpose. PCR-based assays analyzing polymorphic short tandem repeat (STR) markers are shown to be sensitive and rapid method. The intent of the present study was to test this approach for the quantification of mixed chimerism using six to twelve STR assay after boon marrow transplantation. The feasibility of this assay and the accuracy of quantitative results were tested using serial cell mixtures of unrelated individuals. Sequential analysis of individual chimerism status was performed in 63 patients who underwent BMT in Shariati Hospital, Tehran, Iran. Mixed chimerism (MC) was found in 12 of the patients from four weeks till nine months. Using the STR-PCR, discrimination between donor and recipient was possible in all patients analyzed (n = 53) except for one patient who was homologous even for his HLA genotypes with donor genotype. This procedure allows rapid and sensitive quantification of mixed chimerism after boon marrow transplantaion.

P0151. Chromosome imbalances in oligodendroglial tumors detected by Comparative Genomic Hybridization

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Morphologic criteria for diagnosing and classifying oligodendroglial tumors remain the gold standard. Nevertheless, the addition of molecular approaches such as LOH, and Comparative Genomic Hybridization (CGH) can provide a means of arbitrating difficult or borderline cases, and establishing objective, reproducible standards, which will be tested prospectively for their ability to predict prognosis and responsiveness to therapy. Here we report a study on 25 oligodendroglial tumors (7 well-differentiated oligodendrogliomas, 16 anaplastic oligodendrogliomas and 2 oligoastrocytomas) analysed by CGH. Losses of 1p and 19q, known as common markers of oligodendroglial tumors, were observed in 36% (9/25) and 68% (17/25) of cases respectiveley and 32 % (8/25) of the tumors displayed both losses. These 8 tumors with 1p/19q losses have other abnormalities, except one case. The most prevalent deviations associated with this 1p/19q losses were deletions of chromosome 22 (3/8) and gains of the region 13(q21qter) (4/8). Loss of 9p was essentially restricted to anaplastic ooligodendrogliomas (4/16) and occured in tumors with intact 1p or 19q. Interestingly, six tumors (24%) showed gain of chromosome 7 associated in 3 cases with an amplification of the EGFR region (7p11), and loss of chromosome 10, alterations known to be preferentially involved in the progression of astrocytic tumors. This study confirms previous reports and shows that oligodendroglial tumors carry heterogeneous genetic alterations . In addition, these findings suggest that genes localized to these common chromosomal regions play a role in the tumorogenesis of oligodendrogliomas.

P0152. Detection of Illegitimate Rearrangements within the Immunoglobulin Light Chain Loci in B-cell Malignancies.

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Translocations involving the immunoglobulin loci are recurring events of B-cell oncogenesis. However, only a minority of translocations involves the immunoglobulin light chain loci; the kappa light chain (IGK) located at 2p11.2 and the lambda light chain (IGL) located at 22q11.2. We characterized clones from bacterial artificial chromosomes (BAC) libraries, spanning the IGK and IGL loci, for detection of illegitimate rearrangements within the loci by fluorescence in situ hybridization (FISH). Within the IGL region we have identified six end sequenced probes (22M5, 1152K19, 2036J16, 3188M21, 3115E23, and 274M7) covering the IGL variable (IGLV) cluster and two probes (165G5 and 31L9) covering the IGL constant (IGLC) cluster however within the IGK region five probes (479P12, 969D7, 316G9, 122B6, and 2575M7) have been identified covering the IGK variable (IGKV) cluster, and one probe (1021F21) covering the IGK constant (IGKC) cluster. A series of 25 cell lines of different origin have been analyzed for the presence of a translocation involving the immunoglobulin light chain by dual color FISH where the split of the variable cluster and the constant cluster indicated a translocation.
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¤ The two authors have contributed to the work equally.

P0153. Cytogenetic Profiling Could be an Adjunct to Differential Diagnosis and Prognosis of Unknown Primary Tumors

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Unknown primary tumors (UPT) constitute an entity, with great clinical and biological interest. The patients' clinical features are heterogeneous and their prognosis is very difficult to predict.
The present study aims to 1.identify UPT-associated cytogenetic aberrations, 2.evaluate the efficacy of cytogenetic analysis in differentiating metastatic carcinomas from lymphomas and sarcomas, and 3.assess the potential of genetic characteristics in UPT prognosis.
G-banding analysis was performed in surgical biopsies from 20 UPT at diagnosis. In cases with tumor material available after G-banding, CGH and interphase FISH were performed in 10 and 5 cases respectively.
The cytogenetic investigation revealed clonal chromosome aberrations in all, but one, of 18 successfully analyzed samples. In the total series of tumors, the breakpoints 1q21, 6q15, 7q22, 11p12-13, 11q21-22, 12cen, and 17p11-12 were most frequently involved in structural rearrangements, whereas the most common imbalances were losses of 1p, 6q, 8p, 9p, 11p, 11q, and 13q. Which of the above chromosomal sites are important to UPT pathogenesis and harbor genes that might determine the highly aggressive phenotype of this diagnostic entity needs to be further investigated. CGH analysis enhanced the classical cytogenetic findings by revealing imbalances in 7 out of 10 cases, including 2 cytogenetically not informative. Interphase FISH analysis, using locus-specific, break-apart probes for IgH(14q32) and ALK(2p23) revealed a lymphoma diagnosis in 3 UPT, histologically classified as malignant neoplasms.
A preliminary correlation analysis between the cytogenetic profile of the tumors and the patients' survival showed that the increase of karyotypic complexity was associated with decrease of patient' survival.

P0154. Methylation-Associated Transcriptional Silencing of E-Cadherin in Association with beta-Catenin Expression in Sporadic Colorectal Carcinomas.

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We investigated the possibility of an epigenetically associated loss-of-E-cadherin function in SCRCs by examining the methylation status of the e-cadherin promoter by means of the methylation-specific PCR (MSP), in tumour and adjacent normal tissues derived from 63 SCRC patients and correlated it with gene transcriptional silencing, at both the RNA and protein level and beta-catenin mRNA expression. Data were associated with patients' clinicopathological features. A more than two-fold decreased expression of e-cadherin gene was observed in 29/63 carcinomas (46%) versus 11/63 (17.5%) and 23/63 (34.3%) that was found to be increased or unaltered respectively. ICH examination revealed reduced E-cadherin protein expression in 21/63 (33,3%) of carcinomas versus 42/63 (66.7%) with increased E-cadherin expression. Decreased e-cadherin gene expression was significantly associated with E-cadherin ICH detection (P=0.0002) and was paralleled with a decreased beta-catenin expression in 70% of the carcinomas examined (P=0.001). Thirty-four out of 61 cases (50,7%) were reported as hypermethylated in e-cadherin promoter locus versus 27/61 (40.3%) that were found to be unmethylated. The methylation status of the e-cadherin gene promoter was significantly associated with E-cadherin expression at both the RNA and the protein level (P=0.002, P=0.004 respectively). A significant association was observed between E-cadherin ICH detection, lymph node metastasis and/or tumour stage (P=0.007, P=0.01 respectively). In agreement with prior work demonstrating that somatic mutations and loss of heterozygosity (LOH) of e-cadherin gene are rare or absent in the vast majority of SCRCs studied, we have found consistent aberrant methylation-associated decrease of E-cadherin expression suggesting an epigenetically mediated loss-of-E-cadherin function.

P0155. A new frameshift AML1/ETO fusion transcript in a patient with t(8;21) positive acute myeloid leukaemia

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Acute myeloid leukaemia (AML) is a heterogeneous disease, with individual cases showing variability in clinical presentation, blast cell morphology, therapeutic response and long-term prognosis. One of the most frequent cytogenetic abnormalities in AML is t(8;21)(q22;q22), found in approximately 10-15% of the cases. The t(8;21) fuses the AML gene to the ETO gene also identified as MTG8 (myeloid translocation gene on chromosome 8), generating predominant PCR products of a constant size (260bp), corresponding to an in-frame fusion of AML1 exon 5 to ETO exon 2.
We present one AML patient in wich an abnormal AML1-ETO fusion transcript was observed by RT-PCR. The sequence of this purified product revealed a 50 bp frameshift deletion in exon 2 of the ETO gene. The loss of 50 bp originates a disruption of the reading frame of this transcript creating a stop codon 48 aa downstream. As a consecuence of this deletion, the expected protein will be a truncated form. Due to the fact that: a) AML breakpoints are clustered between exon 5 and exon 6, and ETO breakpoints are located upstream of exon 2 and, b) the deletion is located in exon 2 of the ETO gene, the structure of this truncated fusion-protein will included only 31 aa of the ETO gene.
AML/ETO has been shown to fuction as a transcriptional activator that is critical for the tissue-specific expression of a number of haemopoietic specific genes. This case shows that most ETO sequence may be dispensable in AML/ETO+ leukemias.

P0156. RET oncogene mutations in Serbian patients with thyroid medullary carcinoma

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Medullary thyroid carcinoma (MTC) is rare malignant disease with poor prognosis. Point mutations in the RET oncogene are the hallmark in the molecular pathogenesis of this disease. This malignancy presents with sporadic and inherited etiology. In the view of the fact that genetic penetrance is close to 100%, early detection of inherited mutations in the RET oncogene offers the basis for prevention of MTC. This report describes initial efforts to establish genotype/phenotype relations in Serbia. We analyzed DNA from 35 tissue specimens of 21 patients histopathologically diagnosed as MTC and from 6 blood specimens of their relatives. Namely, two patients provided informed consent for the gene analysis of family members where heritable nature of mutated RET was confirmed. RET gene (exons: 10, 11, 13, 15 and 16) was scanned for mutations by single strand conformation polymorphism (SSCP) and heteroduplex analysis (HD). Mutations characterized by direct cycle sequencing and restriction fragment length polymorphism (RFLP). Germline mutations were detected in 4 patients from distinct families. These mutations were: TGC618AGC, TGC629CGC, ATG918ACG and TGC634CGC. Four patients were characterized by somatically acquired mutations in the RET oncogene. One of sporadic cases presented with mutation in exon 10, TTG610TCG, (Leu→Trp) not previously described in literature. This initial research effort is currently being extended to population wide scope.

P0157. How well do the old and new criteria identify Li-Fraumeni families?

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Li-Fraumeni syndrome (LFS) is a dominantly inherited cancer predisposition caused by mutations in the p53 gene. The cancer spectrum in LFS is broad complicating the identification of families. Recently Chompret et al. (2001) introduced new LFS-criteria based on the occurrence of narrow spectrum tumors including breast, sarcoma, brain, and adrenocortical cancer. The criteria include: 1. A proband with a narrow spectrum tumor <36 years with a 1° relative with a narrow spectrum tumor <46 years (other than breast cancer, if the proband had this) or with multiple tumors. 2. A proband with two narrow spectrum tumors, the first of which diagnosed <36 years. 3. A proband with adrenocortical carcinoma regardless of the age or family history. The more stringent classical criteria include: a proband with sarcoma <46 years, a 1° relative with cancer <46 years, and another 1° or 2° relative with cancer <45 years or sarcoma at any age. We used both criteria to categorize 14 families with a clinical LFS-suspicion. p53 mutation screening had also been performed. Two mutation positive families fulfilled both criteria. Ten families, of which five were mutation positive, fulfilled only the new criteria. Two mutation negative families fulfilled neither of the criteria, although one proband had both breast cancer and sarcoma, and the other had sarcoma and a sister with breast cancer. None of these cancers were, however, diagnosed <36 years. In our series, the specificity and sensitivity for the old criteria were 100% and 29%, and for the new 58% and 100%, respectively.

P0158. Molecular cytogenetic profile of invasive transitional cell urinary bladder cancer determined by comparative genomic hybridisation

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We analyzed chromosomal abnormalities (CA) in 25 tumor specimens originating from 25 Bulgarian patients with invasive transitional cell cancer of the urinary bladder by comparative genome hybridization (CHG). A total of 168 CA were detected in 21 cases (6,7 aberrations/case), the remaining 4 being normal. The distribution of the CA (gains-CG, loses-CL, whole chromosome gains-WCG) for the whole group and pT1/pT2-4, and G2/G3 subgroups are given in the table.

	PT1	pT2-4	G2	G3	Total
CG	56 (3,7/ case)	64 (6,4/ case)	83 (4,6/ case)	37 (5,3/ case)	120 (4,8/ case)
CL	5 (0,3/ case)	21 (2,1/ case)	22 (1,2/ case)	4 (0,6/ case)	26 (1/case)
WCG	12 (0,8/ case)	10 (1/case)	12 (0.7/ case)	10 (1,4/ case)	22 (0,9/ case)
Number CA	73 (4,8/ case)	95 (9,5/ case)	117 (6,5/ case)	51 (7,3/ case)	168 (6,7/ case)
Number cases	15	10	18	7	25

Individual case analysis of CG demonstrated that the most frequently affected regions were 1p, 1q, 2q, 3p, 7q, 16p, 17q, 19p and 19q (representing 51,4% of all chromosome gains). CL on 9, 6 and 10 represented 46% of all chromosome losses. WCG were found most frequently for chromosome 19 (5 cases).
Multiple case analysis included combined bar analysis and pool chromosome analysis. Combined bar analysis demonstrated that the minimal overlapping regions of CG were 1p36, 1q12.11-12.12, 3q12-13, 3q27, 12q12.2-12.4, 19p12 and 19q12. The 3q12-13 was characteristic for the pT1 whereas 1p36 for the pT2-4 group.
Multiple case CGH analysis (pool chromosome analysis) demonstrated significant CG at regions 1q12.11-12.13, 16q11-12 and 19p for the whole group, 1q12.11-12.13, 6q14.5-16.3 and 19p for the pT1, 19p for the pT2-4 and G2 group, and 2q12.3-14.1, 8q21.31-ter, 11q13.1-14.1, 11q14.31-ter, 12q14.2-ter, 13q32-33, 15q21.11-ter, 16q, 18q21-23.1, 19q, 20q11.41-45, 21q22.21-ter for the G3 subgroup.

P0159. Frequency of frameshift alterations in polynucleotide repeat-containing genes in HNPCC tumors

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DNA sequences made of mono-, di- and trinucleotide sequences are prone to replication errors and thus constitute mutational hot spots. This is well illustrated by the occurrence of DNA microsatellite instability in tumors from patients affected with hereditary non-polyposis colorectal cancer (HNPCC), resulting from a defect in a gene that controls post-replicative DNA mismatch repair (MMR). We analysed 10 tumors (9 colorectal and 1 ovary carcinomas) from HNPCC patients carrying a germline mutation in the MMR gene MLH1. For each tumor, error rates were measured by sequencing 20 to 50 cloned amplicons from 4 genes involved in either cell proliferation or apoptosis. The polynucleotide tracts selected consisted in a 10 A coding repeat in the TGFbRII gene, an 8 G coding repeat in the BAX gene, a 7 A coding repeat in the CASP1 gene, and a 7 CCA repeat in the 3'-UTR of the APP gene. Substantial inter-tumors variations were observed in the pattern of alterations, with error rates varying between 12 and 80% for TGFbRII, 2 and 84% for BAX, 0 and 30% for CASP1 and 0 to 18% for APP. In contrast with previous results obtained not from single molecule analysis, the BAX error rate did not exceed 20 % in 9 tumors. High error rates in more than one gene in a same tumor suggested additive selective effects from different alterations. These data on somatic frameshifts in specific genes may contribute to better tumor classification and outcome prediction.

P0160. VHL gene mutations in Czech and Slovak patients with VHL disease and sporadic hemangioblastoma

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The Von Hippel-Lindau syndrome is a dominantly inherited familial cancer syndrome with an incidence of 1 in 53000 to 85000 persons, predisposing to retinal, cerebellar, and spinal hemangioblastoma, renal cell carcinoma, pheochromocytoma and pancreatic tumors. The disease is caused by germ-line mutations in the VHL tumor suppressor gene. Between 1996 and 2001, sixteen unrelated probands fulfilling clinical criteria of VHL disease and seven patients with sporadic hemangioblastoma (2 retinal, 5 cerebellar) were included to the study. Detection of mutations in the VHL gene was performed by Southern blot analysis, DGGE of exons 2 and 3, and DNA sequencing of exon 1 in all samples and of exon 2 or 3 in the DGGE-positive samples. Germ-line mutations of the VHL gene were identified in 13/16 (81%) patients with VHL disease and in 2/7 (29%) patients with sporadic hemangioblastoma. Among the identified mutations one mutation was novel (Y112S) and 14 mutations have been already described by others (P25L, S65L, S65W, F76del, S80N, L101R, G132X, R161X, 2x R167W, 3x R167Q, 10kb genomic deletion of exon 3). Except the P25L substitution, which could represent a polymorphism, the mutations are pathogenic. Moreover, one rare variant, IVS2+8c>t, was also identified in one healthy individual. In seven families, the identification of mutation in proband was followed by presymptomatic DNA testing in 29 relatives at risk. Negative results of mutation analysis in 3 patients with familial VHL disease require further study. This work was supported by the Grant Agency of Charles University (Grant No. 36/1996).

P0161. Cyclin D1 Cd242 G-A Polymorphism is not a Risk Factor for Colorectal Cancer in Patients from The Republic Of Macedonia

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Cyclin D1 is involved in the regulation of the transition from G1 to S phase of the cell cycle and is often over-expressed in tumors. A G→A polymorphism at CD242 of the CCND1 gene was implicated as an important risk factor for development of colorectal cancer at a younger age, both among HNPCC and sporadic cases. We evaluated the prevalence of the CCND1 polymorphism using RFLP-PCR among 136 colorectal cancer patients and 170 normal controls from Macedonia. The allele frequencies and the distribution of different genotypes of the case subjects was similar to those of the controls (A/G allele 0.55/0.45 and 0.51/0.49, respectively; AA/AG/GG genotypes 33.1%/44.1%/22.8% and 25.3%/51.2%/23.5%, respectively). No differences of allele frequencies and genotype distribution were observed when cases were grouped by age, Dukes stage, localization, histological type, MSI, p53 and 18q status ($p>0.05$). The observed differences in the CCND1 genotypes between colorectal cancer patients from Macedonia and the USA are similar to the differences that were observed for the transforming growth factor b-type I receptor polymorphism (Stefanovska et al., Cancer Res., 61:8351, 2001), and strongly suggests that certain environmental factors influence colorectal cancerogenesis through multiple mechanisms.

P0162. The role of the H-*ras* oncogene in neurometabolism: Do depression and cancer stem from a common etiology?

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An association between depression and cancer has long-been recognized. Essentially, the prevailing hypothesis is that neurometabolic disturbances inherent in depression effect a change in endocrine and immunological systems making one at greater risk for cancer. More recently, molecular epidemiologists have postulated that the linkage between depression and later cancer onset might be genetic in nature. Specifically, the H-*ras* oncogene has recently been implicated as a source of dysfunctional neurometabolism, presenting as an alternative hypothesis to the prevailing view that cancer results from a weakened immune system. Essentially, it has been

theorized that dysregulation of this cancer gene results in impaired serotonin and dopamine synthesis secondarily. However, whatever the cause of depression/cancer comorbidity, studies have failed to confirm a definite link between depression and later onset of cancer. I expect that two confounding variables exist within the most recent depression/cancer correlational studies. First, these studies have failed to incorporate the growing body of literature delineating the role of the H-*ras* oncogene in neurometabolism. It is plausible that a dysfunctional H-*ras* gene may be the causal link between depression and cancer. If so, longitudinal studies seeking to correlate depression and cancer should parse out cancers known to be associated with the H-*ras* gene. Second, existing studies fail to employ an accurate classification and diagnosis of depression. My hypothesis is that a depression/cancer correlation is caused by a dysfunctional H-*ras* oncogene which acts *primarily* to disrupt neurometabolism, the serotonin and dopamine biosynthetic pathways specifically, and *secondarily* causes cancer over time.

P0163. Identification of Deoxyribonucleic Acid Copy Number Changes in Larynx Carcinoma by Comparative Genomic Hybridization

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Carcinomas of the head and neck represent 5% of all human cancers, squamous cell carcinoma being the most important group. Head and neck carcinomas including laryngeal carcinoma have been investigated recently by various molecular, cytogenetic, and molecular cytogenetic techniques.
In this study, comparative genomic hybridization(CGH) technique was used to identify DNA copy number changes in 15 paraffin-embedded tissue samples of the larynx carcinoma. Of these patients, 13 were male, and 2 were female. DNA copy number changes were detected in 10 of the 15 patients(66.6%). While 3 of the 10 patients had polyploidy, other 7 patients were found to have gains and losses on different chromosomes. 5 cases had normal CGH profiles. The results of the study were compared with literature reported previously and similar findings were detected in chromosomes 5p, 7q, and 18p in larynx carcinoma. Also, loss of chromosome 22q13-qter was found as a novel site in a case with larynx carcinoma.
Although the number of tumor samples investigated is rather low, our results suggest that the chromosomal loci which affect the differentation and progression of the larynx carcinomas can be detected by CGH technique.

P0164. Cytogenetic and molecular response in CML patients on Interferon-α 2b (IFN-α 2b) therapy using conventional cytogenetics and FISH analysis

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Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterised by the presence of Philadelphia (Ph[1]) chromosome resulting from balanced reciprocal translocation, t(9;22)(q34;q11) leading to the formation of *bcr/abl* fusion gene. Studies have shown that IFN-α therapy induces both cytogenetic response (reduction in Ph+ cells) and molecular response (reduction in the *bcr/abl*+ cells) in a significant proportion of CML patients thereby improving their prognosis and survival. To the best of our knowledge, no published reports are available from India using molecular methods for evaluation of minimal residual disease.
The present study was conducted to evaluate the cytogenetic and molecular response in CML patients on Interferon-α 2b (IFN-α 2b) therapy. Sequential cytogenetic analysis was done using standard methods in 45 CML patients on IFN-α 2b therapy up to a variable period of 3 years. Further dual colour Fluorescence In Situ Hybridisation (FISH) analysis using specific probes for *bcr* and *abl* genes was done to assess the molecular response. Complete cytogenetic response (CCR) was observed in 8 patients. Of these 8 patients in CCR, 4 were negative for the *bcr/abl* fusion gene implying a complete cytogenetic and molecular response while the remaining 4 showed *bcr/abl* fusion signals representing residual disease. Thus the present study stresses on the need for sequential cytogenetic and molecular analysis in CML patients on therapy. The importance of using FISH on interphase nuclei and poorly spread metaphases that cannot be analysed using conventional cytogenetics is also highlighted.

P0165. High frequencies of primary multiple melanomas in families with CDKN2A mutations.

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About 10 % of melanoma is inherited in an autosomal dominant fashion with variable penetrance and 50-80% of the families are linked with 9p21. CDKN2A, located in 9p21, consists of three coding exons and encodes the cell cycle inhibitor, p16. This protein plays a role as a negative regulator of the cyclin D1/CDK4/p16/pRb signaling pathway, the major growth control pathway in the cell cycle. In our study, on a wide sample of melanoma-prone families, we found 8 pedigrees in which a CDKN2A mutation was evidenced, some already described in literature and some as a new mutation (Pro48Thr, ivs1+2(T-C), 201delC). Analyzing clinical data of these 8 families we observed that in 6 (75%) it was present at least one multiple primary melanoma (MPM). The presence of a MPM in a so large amount of the mutated cases brings us to consider CDKN2A mutations as responsible to a high constitutional risk for melanocytes transformation. For this reason, we think relevant to include CDKN2A mutational screening in all the families in which there is one patient with MPM, even if not familial.
In addition in 2 out of our 8 families a larynx carcinoma was also present, supporting the hypothesis of a related risk for this tumor in CDKN2A mutation carriers.
Al last, we also observed, in 5 out of these 8 pedigrees, the presence of only two melanoma patients suggesting the opportunity to extend CDKN2A mutations analysis also to families with less then three affected relatives, at least in Italian population.

P0166. Mutational and expression analysis of the NF1 gene argues against a role as tumor suppressor in sporadic pilocytic astrocytomas

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Children with Neurofibromatosis type I (NF1) have an increased risk for developing pilocytic astrocytomas (PAs). LOH studies demonstrate frequent loss of the NF1 gene in NF1-associated PAs. Further, it has been demonstrated that loss of neurofibromin in a NF1-associated PA is associated with elevated Ras-GTP levels. However, conflicting results on the role of the NF1 gene in the development of sporadic PAs have been reported. Therefore, we investigated 14 sporadic PAs for NF1 mutation and for LOH within the NF1 locus. The protein truncation test, which identifies approximally 80% of the mutations found in NF1 patients, failed to detect NF1 mutations in 10 analyzed tumors. LOH analysis was unable to reveal evidence for disruption of the NF1 gene in 11 informative cases. The GTPase-activating domain of the NF1 gene is expressed in two isoforms. Using real-time PCR we investigated the ratio of the isoforms in 14 sporadic PAs and compared it to the ratios in normal adult tissues, glioblastomas and neuroblastomas. In accordance with previous reports we found marked predominance of the type II transcripts in PAs as well as in two glioblastomas and in all analyzed adult tissues including brain. In contrast, marked predominance of the type I transcripts were observed in neuroblastomas. Our results argue against a role of the NF1 gene as tumor suppressor in sporadic PAs and suggest that the predominant expression of the type II NF1 transcript in PAs reflects the differentiation stage of the cells rather than being a response to elevated cell proliferation.

P0167. Characteriziation of differentially expressed candidate genes associated with gynaecological tumors

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More than 600 candidate genes were identified as differentially expressed in gynaecological tumors by “in-silico” approaches and fifty of them, considered as either putative tumor suppressor genes or oncogenes, were selected for further analysis in sporadic gynaecological tumors by the GCC (Gynaecological Cancer Consortium).
To confirm the electronic Northern data, seven putative TSGs were investigated by hybridization of cancer arrays (Clontech cancer profiling arrays) against gene-specific probes. Two genes (designated bn39, bn40) were found to be expressed lower in up to 80% of breast or ovarian cancer samples compared to matched corresponding normal tissues, respectively. These data could be supported by real time PCR (TaqMan) performed in matched tissue samples collected at our hospitals.
For each of the two determined TSG candidates, 20 LOH positive tumor samples were screened for mutations by the DHPLC technique. No functional mutations were found in one downregulated candidate gene, only one frame-shift mutation and one missense mutation were detected in the other assumed TSG (bn40). To characterize three putative oncogenes, expression was analysed by hybridization of cancer arrays and assumed amplification was determined by real-time PCR or quantitative differential PCR. One candidate gene (bt11) was shown to be frequently over-expressed especially in ovarian cancer while gene amplification could not be shown.
Conclusions: About 30% of the nominated candidate genes identified by database screening were shown to be differentially expressed in-vivo. Down-regulation of gene expression in cancer tissues might be explained by epigenetic inactivation/activation mechanisms rather than mutations or gene amplification.

P0168. HNPCC - two different entities in regard of mutation analysis and clinical phenotype

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HNPCC is caused by heritable mutations in the DNA mismatch repair genes.
For 254 patients that fullfilled one of the Bethesda Kriteria 1-7, mutation analysis for hMLH1, hMSH2 and hMSH6, microsatellite analysis and immunohistochemistry for hMLH1, hMSH2 and hMSH6 was performed. 25 pathogenic mutations and 24 missense variations were found. Data concerning the sensitivity of microsatellite analysis and immunohistochemistry will be presented.
The cohort included 51 families fullfilling the Amsterdam Criteria. First, 11 patients with MSI-H tumors and truncating mutations. Second 5 patients with MSI-H tumors without mutation in hMLH1, hMSH2 and hMSH6. Third 7 patients with MSI-H tumors and missense mutations in hMLH1 or hMSH2 or truncating mutations in hMSH6. Fourth, 6 patients with MSS or MSI-L tumors with suspected missense mutations in hMLH1 or hMSH2 or truncation mutatins in hMSH6 and fith, 22 patients with MSS tumors without mutations in hMLH1, hMSH2 or hMSH6.
Clinical and molecular data of the first and the fourth group revealed differences concerning age of onset, tumor spectrum within the families, tumor localisation and pathohistological features. There is an earlier age of onset and a broader spectrum of tumors in the families of group 1. Tumors are more right sided and more frequently show the typical HNPCC-associated histopathological features. In hMSH2 endometrial cancer seems to cluster with mutations in exon13. Life expectancy of female mutation carriers is increased compared to male mutation carriers.These data point towards the existence of at least two entities of hereditary colon cancer other than FAP.

P0169. Frequent epigenetic silencing of the CpG island promoter of RASSF1A in thyroid carcinoma

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LOH of chromosome 3p21 is one of most frequent alterations in solid tumors. RASSF1A-isoform is epigenetically inactivated in a variety of human primary tumors. We investigated expression and methylation status of RASSF1 gene in 38 primary thyroid tumors (1 PDTC, 5 MTC, 10 FTC, 9 UTC, 13 PTC) and 9 thyroid cancer cell lines. In all cell lines the RASSF1A promoter CpG-island was completely methylated and expression was absent. Treatment of these cell lines with DNA methylation inhibitor 5-aza-2-deoxycytidine reactivated the transcription of RASSF1A. In 71% of primary thyroid carcinomas the RASSF1A-promoter was hypermethylated. Methylation frequency was higher in aggressive forms of thyroid carcinoma (80% of MTC, 78% of UTC and 70% of FTC) compared to 62% in more benign PTC. RASSF1A-inactivation was detected in all stages of thyroid carcinoma scored by pTNM-classification. Additionally, we analyzed the methylation frequency of CpG-island of cell cycle inhibitor p16INK4a in the same thyroid tumors. The p16-gene was inactivated in 56% and 25% of cell lines and primary tumors, respectively. p16 methylation was detected in 56% of UTC, in 10% of FTC and in 25% of PTC, but not in MTC. In UTC, which belongs to the most aggressive carcinomas in humans, the most common combined inactivation of RASSF1A and p16 was detected. In general, 90% of tumors with p16 inactivation were also silenced for RASSF1A expression. However, RASSF1A hypermethylation was detected three times more frequently in thyroid cancers. Thus, RASSF1A inactivation may play a crucial role in the malignancy of thyroid carcinoma.

P0170. Analysis of genomic copy number and expression of genes in the chromosomal band 8q11 in hepatoblastoma

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Recently, the correlation of comparative genomic hybridization (CGH) results with clinical data in a large series of hepatoblastomas, has uncovered that gain or amplification of chromosomal 8q material is associated with poor prognosis of this highly malignant childhood tumor. The minimal amplified region was defined to chromosomal bands 8q11.2-q13. In an attempt to identify hepatoblastoma-related genes in this region we implemented a strategy that combined restriction landmark genomic scanning (RLGS) and a genomic copy number assay based on real-time PCR. RLGS analysis uncovered six chromosome 8 derived fragments amplified in one hepatoblastoma. Virtual genomic scann, a novel informatic tool for sequence prediction of RLGS fragments, identified the sequence of five of these fragments. Thus, the critical region was defined to chromosomal band 8q11 extending at least between the two genes, SOX17 and Lyn. Four microsatellite markers and genes located between or adjacent to these genes as well as four control markers were selected for genomic copy number analysis. Seven of 19 tumors investigated (37%) showed gain or amplification in this region, including three tumors in which a gain was undetectable by CGH analysis. The expression of five genes and ESTs, located within the newly defined minimal amplified region was assayed by real-time RT-PCR in 10 hepatoblastomas. The gene encoding the transcription factor PLAG1 showed increased RNA expression in all but one hepatoblastoma when compared to normal liver. The possible role of PLAG1 as an activator of fetal growth factor IGF2 in hepatoblastoma is currently investigated.

P0171. Cytochrome P450 - CYP2D6 polymorphism in head and neck cancer patients

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We investigated the possible association of drug metabolizing enzyme system CYP P450 CYP2D6 and its null alleles (CYP2D6*3, *4, *5, *6, *7, and *8) with incidence of tumors in patients having head and neck cancer (HNC). It is known that persons bearing two null alleles poorly metabolize some common drugs (Poor Metabolizer Phenotype – PM) as well as other foreign and carcinogenic substances. Persons with only one disrupted CYP2D6 gene (bearing one normal and one null allele) are considered to be Intermediate metabolizer phenotype (IM). We genotyped 145 controls, and 42 HNC patients by Multiplex Allele Specific PCR on whole blood DNA. Study results showed allelic frequencies for *3, *4 and *6 alleles (only alleles observed) in controls to be 1.4%, 11.0% and 1.0%, respectively; among them we found 2.1% PMs and 22.8% IMs. In cancer patient's group allelic frequencies for *3, *4 and *6 were 1.2%, 19.0% and 3,6% respectively, and no other alleles were found; among them we found 2.4% PMs and 42.9% IMs. Results of our study showed statistically significant difference for genotype frequencies (Chi-square; p=0.025) and predicted phenotype (Chi-square; p=0.034). IM phenotype showed to be responsible for increased risk to HNC (Odds ratio 2.6; 95%CI= 1.248 - 5.193). To confirm our preliminary findings, further study on a larger group is planned.
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P0172. Glutathione S-transferase polymorphisms influence plasma antioxidants level and oxidative DNA damage.

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Glutathione S-transferases (GST), xenobiotic detoxifying enzymes, involve in defences against oxidative stress and metabolism of plenty carcinogens. Hence the GST polymorphisms are supposed to be significant determinants of individual cancer risk.
We screened 155- middle aged men (51 smokers and 104 non-smokers) for GSTT1null, GSTM1null and GSTP1b polymorphisms and compared them with parameter of oxidative stress at the level of oxidative DNA damage measured in lymphocytes, and with plasma antioxidants level.
Smokers had on average significantly lower levels of plasma antioxidants and higher amounts of oxidised purines and pyrimidines measured in lymphocyte DNA. The observed genotype frequencies were represented as follows: 48% GSTM1null, 21% GSTT1null and 11% GSTP1b/b. The GSTT1 null genotype was associated with decreased Vitamin C concentration compared to GSTT1+ genotype, while Vitamin C was higher in GSTM1 null compared with GSTM1+. The homozygous GSTP1 a/a genotype was associated with significantly higher levels of GST activity measured in lymphocytes, in comparison with the b/b genotype. Using multifactorial statistical analysis significant interactions were found between smoking, GSTP1 genotype, plasma Vitamin C, and purine base damage in lymphocyte DNA. Vitamin C concentrations were substantially higher in b/b non-smokers compare with b/b smokers, whereas this phenomenon was not observed neither in a/a nor a/b groups. The b/b smokers had on average about twice as much oxidised purine base damage as the non-smokers with that genotype, and higher levels than the other smokers. In contrast, the link between smoking and oxidised pyrimidines in DNA was seen only in the GSTT1 null group.

P0173. Carney triad in a patient with balanced translocation t (1;19) (p11-13;p11-12).

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The Carney triad is a rare association of gastric epithelioid leiomyosarcoma, functioning extra-adrenal paraganglioma and pulmonary chondroma, affecting specifically young individuals. It probably has an autosomal dominant pattern of inheritance. However, the molecular basis of this unusual syndrome has not yet been elucidated. We report a new case of incomplete Carney triad in a female who presented first with a pulmonary chondroma at age 21. At age 27, she developed a primary gastric leiomyoblastoma. Seven years later, a total gastrectomy was performed following the discovery of two additional gastric tumors. Intriguingly, karyotypic analysis of PHA-stimulated peripheral blood lymphocytes revealed an apparently balanced translocation t(1;19) (p11-13;p11-12). This chromosomal abnormality seemed to be inherited from her phenotypically normal father. Possible mechanisms whereby such a familial translocation could have a pathogenic effect in our patient include a chimeric fusion gene generated by a complex rearrangement, a de novo duplication of a protooncogene or a de novo microdeletion in the translocated chromosomes disrupting a putative tumor suppressor gene at the breakpoints. This is the first report of a cytogenetic abnormality associated with the Carney triad. The translocation breakpoints in this patient may become candidate regions for susceptibility genes causing this uncommon disorder. Further genetic investigations are carried out by Fluorescent in situ hybridization (FISH) to map potential genes in the 1p11-13 breakpoint region, interestingly containing the *N-RAS* and *notch2* genes.

P0174. Hypermethylation of the 5´ promoter region represses Caveolin-1 gene expression in a human prostate cancer cell line

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LOH of chromosomal region 7q31.1 has been implicated in the pathogenesis of many human cancers, including prostate cancer. The genes encoding Caveolin-1 and -2 are localized at 7q31.1. The Cav-1 promoter contains several CpG dinucleotides of which four are methylated in two human breast cancer cell lines. They fail to express Cav-1 mRNA, suggesting an epigenetic mechanism of Cav-1 gene regulation in these cell lines. We´re investigating the role of Cav-1 in prostate cancer by investigating the expression of Cav-1 in human cell lines derived from normal prostate and prostate cancer. Our findings show that Cav-1 expression is absent from the cell line LNCaP on RNA and protein level. To test the hypothesis that DNA methylation in the promoter correlates with down-regulation of Cav-1 gene expression, we determined the methylation status of additional prostate cell lines. A minimal promoter of Cav-1 contains seven CpG sites and we found a very heterogeneous methylation profile at four of these. Only the promoter region of LNCaP showed almost complete methylation. The functional importance of these CpG sites was demonstrated by an in vitro reporter gene assay which revealed that the cav-1 promoter activity in vitro is regulated by methylation of four CpG sites within its minimal promoter region. We conclude therefore that repression of the Cav-1 gene in LNCaP cells is due to DNA methylation. Furthermore, results from bandshift assays demonstrated that a yet unknown methyl-CpG-binding protein interacts with the methylated Cav-1 promoter region examined and that this protein is different from MeCP2.

P0175. PRUNE and NM23 protein interaction: possible implications in Neuroblastoma.

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We report here a functional characterization of prune protein and its possible correlation with Neuroblastoma cancer. A newly identified phosphodiesterase
(PDE) PDE11A was found to contain a catalytic site motif equally present in the human prune protein and corresponding to the third DHH motif of prune.
A scintillation proximity assay was performed to investigate prune phosphodiesterase activity both on transiently transfected COS-7 crude extracts and on the purified histidine-tagged prune protein produced by the Baculovirus expression system. We demonstrate that prune is able to act as a phosphodiesterase preferentially on cAMP substrate. Furthermore, prune is able to interact with NM23-H1, an antimetastatic protein but its interaction is impaired with NM23-H1S120G, a mutation associated with advanced stages of Neuroblastoma. By in vivo co-immunoprecipitations and interaction mating assays we demonstrate the interaction between nm23-H2 and a series of described nm23-H2 protein mutants.
PRUNE protein is predominantly a cytoplasmic protein. By

immunofluorescence experiments we investigated prune and nm23 localization in SK-N-SH, SK-N-BE, SH-5YSY and IMR-32 Neuroblastoma-derived cell-lines. A prune predominant nuclear localization was observed for the first time. Expression of prune protein was examined by immunohistochemistry analysis on paraffin embedded tissues from 5 Neuroblastoma affected patients revealing high protein expression
in the nucleus and its association with poor outcome. We are preparing stable clones of SH-5YSY cells by transfection (pBABE retroviral vector) of prune cDNA in order to study the growth properties of the stabilized cells and isolate other putative prune nuclear interactors by mono-dimensional SDS-page analysis and Mass Spectrometry.

P 3. Clinical Genetics and Dysmorphology

P0176. Single central incisor, pyriform aperture stenosis, midfacial hypoplasia and limb abnormalities: a new syndrome?

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Solitary median maxillary central incisor (SMMCI) has been reported as an isolated abnormality or in association with other systemic abnormalities including pituitary insufficiency, nasal pyriform aperture stenosis and holoprosencephaly. SMMCI can also be a feature of recognized syndromes or chromosomal abnormalities. SHH and SIX3 mutations have been reported in patients with SMMCI.
We report on an infant with a previously undescribed pattern of malformations including brachycephaly, proptosis, midfacial hypoplasia, SMMCI, abnormal ears, arachnodactyly, partial cutaneous syndactyly and joint contractures. CT scan revealed pyriform aperture stenosis. At the age of eleven months, the patient has developmental delay and suffers from seizures.
Laboratory investigations were within normal limits. Roentgenologic skeletal survey was normal apart from the finding of 11 pairs of ribs. CT scan of the brain was normal. BAER examination revealed hearing loss on the left side. Chromosomal studies including high-resolution chromosomal analysis showed a normal female karyotype. Sequencing of the exons IIIa and IIIc of FGFR2 did not reveal mutations.
We suggest that this combination of anomalies constitutes a unique syndrome. Searching for mutations in the genes responsible for the development of the midline structures should provide a greater understanding of the mechanisms underlying the development of this unique combination of abnormalities.

P0177. Further delineation of Serpentine fibula-polycystic kidney syndrome

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Majewsky et al. (1993) reported on female with serpentine fibulae and reviewed one of the Dereymaeker et al. (1986) and Exner (1988) patients, believing that they represented a rare condition named as Serpentine fibula-polycystic kidney syndrome (SFPKS) characterized by growth retardation, abnormal face, hirsutism, short neck, elongated serpentine fibulae, metatarsus adductus, deafness, normal intelligence and polycystic kidneys. We reported on a sporadic case of a 8-year-old woman with SFPKS brachycephaly, thin upper lip with down turned corners of the mouth, wide nasal tip, long and flat philtrum, dysplastic and dorsally rotated ears, large and short neck, flat chest, widened and prominent distal regions of her arms, deafness, language disturbances and normal intelligence. X-Ray skeletal survey showed: bathrocephaly, sclerosis of mastoids, absent frontal sinuses, diastasis of skull sutures, prominent parieto-occipital synchondrosis, wormian bones, scoliosis, increased anterior height of the lumbar vertebral bodies with abnormal pedicles and reduced intervertebral distances, elongated and serpentine fibulae. She also had multiple polycystic kidneys and an interventricular communication. Similarities between Melnick-Needles (MNS), Hajdu-Cheney (HCS) and SFPKS syndromes were reported, however MNS is rather a skeletal dysplasia rarely presenting ureteral abnormalities. Peculiar facies, marked growth retardation, webbed neck, polycystic kidneys, serpentine fibulae, and metatarsus adductus is typical of SFPKS. Both MNS and SFPKS do not present acro-osteolysis as HCS does. Only six cases were reported and further patients should be identified in order to reinforce the phenotypic spectrum of SFPKS and also to clarify if MNS, HCS and SFPKS are distinct entities or allelic disorders.

P0178. Evidences of autosomal recessive inheritance in unifocal subtype of fibromuscular dysplasia of the renal arteries.

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Fibromuscular dysplasia (FMD) is a group of nonatherosclerotic, noninflammatory occlusive diseases that most commonly involve the renal and carotid arteries whose etiology remains unknown. Dominant inheritance with variable penetrance was demonstrated. Dysplasic stenoses can be multifocal, unifocal and tubular. The most common manifestation of renal artery FMD is renovascular hypertension, between 30 to 50 years.Its prevalence is underestimated due to many FMD cases with normotensive or asymptomatic hypertensive patient remain undiagnosed. We report on three sibs with unifocal FMD renal arteries, two of them with bilateral affected arteries associated to congenital cardiac abnormalities. Interestingly, all presented an early onset of renovascular hypertension (2y-3y)with normal renal function. Only one with unilateral renal stenosis is still alive at 5 years old, after surgical correction. The others witj bilateral affected renal arteries died at about 3 years old. The parents were normotensive and although there was no consanguinity, we thought that a recessive pattern of inheritance should be considered in unifocal FMD of the renal arteries.

P0179. Calcification of the basal ganglia

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Calcification of the basal ganglia was observed in more than 30 medical conditions including infections, trauma and hemorrhage and many genetic syndromes. Diverse neurobehavioral and psychiatric, manifestations have been linked to this disorder. However, its etiology remains obscure. Eleven cases were investigated who showed calcification of the basal ganglia on computed tomography (CT). Main presenting neurological symptoms were loss of acquired milestones (3 cases), epilepsy (5 cases) and developmental delay (8 cases). Age of onset and course of the disease were variable. Calcification in the basal ganglia was bilateral in most of the cases (8 cases). Globus pallidus was by far the most common site of calcification in the basal ganglia. Further calcification in different parts of the brain rather than basal ganglia was present in (4 cases) cortical, subcortical, white matter or dentate nucleus. Measurements of the size of calcification of basal ganglia and the neurological scoring were constructed. Correlation of the neurological impairment and the size of calcification were non-significant. However, the neurological impairment was significantly inversely correlated with the head circumference ($p<0.002$). In addition, the site of calcification inside the basal ganglia was not pathognomonic. Parenatal consanguinity was documented in 9 cases and positive history of affected family members in 7 cases emphasizing the major role of the autosomal recessive gene in the inheritance in these cases. We represent different phenotypes of this mysterious sign and highlights the importance of computed tomography in verification of calcification to overcome the difficulties in genetic counseling of such cases.

P0180. Primary intestinal lymphangiectasia congenital: report of one case.

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Intestinal lymphangiectasia is characterized by obstruction of lymph drainage from the small intestine and dilated lacteal vessels that distort the villus architeture. Primary intestinal lymphangiectasia probably represents a congenital disorder of lymphatics and is often associated with lymphatic anomalies outside the gastrointestinal tract. Patients with this condition have a picture comparable to experimental thoracic duct drainage with lymphedema, hypogammaglobulinemia, lymphocytopenia, skin anergy and impaired allograft rejection. We reported on a sporadic case from a nonconsanguineous and healthy parents, a 9 months girl with lower leg progressive lymphedema (since 15 days of life), right palpebral ptosis, severe chylousascite and chylothorax, hypogammaglobulinemia, hypoalbuminemia, and lymphopenia. Endoscopy revealed a severe edema and a white-tipped villi appearance with well-circumscribed white plaques of varying sizes throughout the proximal duodenum. Small-intestinal biopsy showed numerous dilated lymphatic vessels within the lamina propria and submucosa. There was no evidence of lymphomatous transformation at the proximal small bowel. Lymphoscintigraphy did not revealed any lower leg lymphatic vessels. Medium-chain triglycerids on dietary and albumin associated with diuretic therapy decreased the enteric protein loss, improving not so much the lymphedema, that became softier. Serum albumin and immunoglobulins levels were increased. No signs of colestasis neither Yellow-nail, Lymphedema-distichiasis, Turner, Noonan or Hennekam syndromes were observed. Primary intestinal lymphangiectasia has been described in association with lymphoma in few cases. Thus, we consider important to establish the definitive diagnosis of this condition and to perform an endoscopic evaluation of the proximal small bowel, in order to detect a lymphomatous transformation.

P0181. AAAS mutation in Triple A syndrome: A case-control study.

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Introduction: Mutation in AAAS gene has been proposed as the underlying mechanism of triple A syndrome. We have studied these mutations in a family in order to determine whether the healthy family member is involved.
Method: A family with involved triple A and double A syndrome as well as alacrimia itself comprised study population. Control group was from the noninvolved members of the family. 10 cc of peripheral blood sample were obtained from each member. DNA was extracted from Buffy coat layer and stored in 4 oC. Sequencing analysis was performed for the exon 10 of each member.
Results: Proband was a 17 years old boy with triple A. He had two sisters; A 19 years old otherwise healthy and a 12 years old with double A syndrome. They have been born of a consanguineous marriage. Aunt of their grandfather was involved by alacrimia according to the family history. Sequencing analysis revealed a single-basepair insertion in exon 10 of the AAAS gene (1-BP INS, 1071T) in proband and the younger sister. Interestingly, we found the mutation in his father which was otherwise healthy. Unfortunately, the grandparents were not alive at the time of the study. Other members of the family showed no mutation in the AAAS.
Conclusion: It seems that 1-BP INS, 1071T which was reported previously in turkey is the responsible gene for the disease in Iran. We propose that the mutation in AAAS alone can not induce triple A in involved cases.

P0182. Spondylothoracic dysplasia (Jarcho-Levin syndrome) and Spondylocostal dysostosis, the confusing vertebral malsegmentation syndromes. Report of six cases

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Jarcho- Levin Syndrome (JLS, Spondylothoracic dysplasia) is the severest form of vertebral malsegmentation syndromes with reduced stature resulting from axial skeleton. The main features are short, immobile neck and small thorax with the patognomonic “crab-like” rib cage associated with multiple vertebral defects, what frequently leads to respiratory problems and death in infancy. Carefully prenatal ultrasound examination during the second trimester should done for subsequent pregnancies. A clinically similar disorders is Spondylocostal dysostosis (SCD). The main features are abnormalities of vertebral segmentation and of the ribs, including multiple hemivertebrae, vertebral clefting and fused, hypoplastic vertebrae, rib fusions and deletions with a non-progressive kyphoscoliosis. Survival is much better and neural tube defects only rarely occur. Cases are sporadic or familial, both recessive and dominant autosomal inheritance has been reported. The identification of genes affecting somitogenesis will be assist better classification. Recently mutations in the recessive form were demonstrated in the Notch pathway gene, DLL3, mapped at 19q13. We describe here six cases of multiple vertebral segmentation defects. Two newborns with the classical features of JLS, both had respiratory problems with “fan-like” chest deformities and one associated with thoracic meningomyelocele and club foot deformity. The remaining four infant presented features of SCD. These had short neck and trunk, different degree kyphoscoliosis and occasional spina bifida. All six patients were sporadic, and parental consanguinity were present by half of them. We believe that appropriate classification of these similar phenotypes will improve molecular research and genetic counselling concerning recurrence risk, management, prognosis and prenatal diagnosis.

P0183. Prenatal Diagnosis Of Dysmorphic Syndromes By Routine Fetal Ultrasonographic Examination Across Europe

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Objectives
Ultrasound scan in the midtrimester of pregnancy is now a routine part of antenatal care in most European countries. The objectives of this study was to evaluate the prenatal diagnosis of non chromosomal dysmorphic syndromes by fetal ultrasonographic examination.
Methods
Data from 20 registries of congenital malformations in 12 European countries were included in the study.
Results
There were 2454 cases with congenital heart diseases, including 104 syndromes, 49% of them were detected prenatally.
1130 cases with renal anomalies including 64 syndromes, 83% of them were detected prenatally, 250 cases with limb reduction deficiencies including 38,12 were syndromes diagnosed prenatally.
Prenatal diagnosis was performed in 7 out of 7 cases with gastroschisis, in 12 out of 14 of cases with omphalocele and in 37,5% of cases with intestinal anomalies (24 out of 64).
There were 553 cleft lip and palate (CL(P)) and 198 cleft palate (CP) 73 recognised syndromes. Prenatal diagnosis was done in 51 CL(P) (53.1%) and 7 CP (13.7%).
Few anencephalic cases were syndromic. Out of 290 cases with spina bifida, 18 were recognized syndromes, 17 of them were diagnosed prenatally. All 11 syndromic encephaloceles were diagnosed antenatally.
Conclusions
In conclusion this study showed that around 50% of the recognized syndromes can be detected antenatally by the anomaly scan. However the detection rate varied with the type of syndromes and with the policy of prenatal screening between countries.

P0184. Polish group of PWS patients - clinical, cytogenetic and molecular investigations

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Introduction: We present results of the clinical, cytogenetic and molecular studies carried out in the group of 77 patients with verified clinical diagnosis of Prader-Willi syndrome (PWS) selected from 202 patients with tentative clinical diagnosis of PWS. **Methods:** Clinical

manifestation, family data, history of pregnancy, parental age and anthropometric traits were analysed and compared in two groups of patients with deletion and mUPD. Cytogenetic analysis was routinely performed using HRBT in all patients. FISH was done in all cases which were diagnosed in our genetic unit. Methylation analysis, gene dosage analysis for *SNRPN* and polymorphism analysis for loci within the PWS/AS region are included in our molecular diagnostic procedure. **Results:** PWS diagnosis was confirmed by methylation test in 77 (38%) patients. Among them deletion was detected in 40 (25%) patients, mUPD in 11 (14,2%) and imprinting mutation in 1 (1.3%) patient. Detection of the molecular defect was impossible in 6 (7,8%) patients because of uninformative polymorphism analysis results. For 19 patients the procedure for the purpose of molecular defect detection is in progress. Detailed comparison of phenotype and anthropometric evaluation will be presented in the group of deletion and non-deletion patients. **Discussion:** Our results of clinical and molecular investigations are comparable with those from published analyses. The numerous group of clinicaly missdiagnosed patients indicated on difficulties in the process of PWS diagnosis. It points to how much still needs to be done to increase practical knowledge of PWS natural history and clinical symptoms among medical doctors in Poland.

P0185. Clinical and cytogenetic analysis of patients with chromsome 18 aberrations.

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We would like to report 12 cases of chromosome 18 aberrations that were evaluated recently in our laboratory. The purpose of the study was to analyze the cytogenetic, clinical and available familial data of these patients. Cytogenetic findings: the GTG banding technique demonstrated in 8 cases the presence of trisomy 18. The remaining karyotypes were the following: 46,XY, r18 and 46, XY, t(4;18). Clinical data: in all cases the family history showed no special disorders. All the infants with trisomy 18 (7 girls and 1 boy -ratio which confirms the female predominance observed in this syndrome) were diagnosed at birth and in all cases the physical examination revealed characteristic phenotypical features: low birth weight, low-set ears, protuberant occiput, characteristic positioning of the hands, limitation of thigh abduction and cardiac malformations. The phenotype of the patient with r18 shared the features of 18p.- and those of 18q- syndrome. Low birth weight, cryptorchidism and micrognathia were the only physical abnormalities observed in a boy with t(4; 18). Outcome: as expected 11 out of 12 infants bearing trisomy 18 died shortly after the birth. It is noteworthy that one girl survived the first year of life which is unusal for the patients with a free homogenous trisomy 18. Infants with aberrations other than trisomy 18 are both alive.

P0186. Phenotype and differential diagnosis of a neonatal Marfan syndrome due to a new FBN-1 exon 25 mutation

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Neonatal Marfan syndrome (nMFS) is the most severe presentation of Marfan phenotypes. nMFS is characterized by flexion contractures, arachnodactyly, crumpled ears, loose redundant skin and visceral anomalies : pronounced atrioventricular valve dysfunction and sometimes ectopia lentis. Death usually occurs within the first year of life from heart failure.
nMFS results from mutations clustering in exons 24 to 32 of the fibrillin-1 gene (FBN-1). Until now, only 19 mutations have been described.
This disorder has to be differentiated from severe lethal Congenital Contractural Arachnodactyly (CCA), caused by mutations in the fibrillin-2 gene (FBN-2). Severe lethal CCA is characterized by a nMFS-like appearance, but there is no ectopia lentis and visceral anomalies are different : cardiac septal defects, interrupted aortic arch and gastrointestinal abnormalities.
We describe a newborn male, born from nonconsanguineous healthy parents. At birth, he presented with a severe respiratory distress. Characteristic features of nMFS and CCA were seen at physical examination. An echocardiogram showed marked prolapse and regurgitation of both mitral and tricuspid valves. Neither gastrointestinal, nor ocular abnormalities were present. Despite vigorous treatment, death occurred at 62 hours from cardiac failure. The visceral anomalies suggested the diagnosis of nMFS. Molecular analysis confirmed this diagnosis with the detection of a new FBN-1 missense mutation at nucleotide 3165 in exon 25 (C1055W).
In conclusion, nMFS and severe lethal CCA can be clinically distinguished by visceral anomalies. The FBN-1 exon 25 mutation in our patient confirmed the diagnosis of nMFS and is in agreement with the previously described genotype-phenotype correlation.

P0186. Bowen syndrome ?

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Case history: Healthy non consanguineous parents, no previous miscarriages.
First pregnancy: Ultrasonogram at 22 weeks revealed severe intrauterine growth retardation and a set of malformations: corpus callosum agenesis, sloped forehead, hypertrophic crystalline lens, microretrognathia with lower maxilla hypoplasia, interventricular septal defect, bilateral pyelic ectasia, hypoplasia in 2nd phalange of both index fingers, malpositioned feet and ambiguous genitalia. Karyotype: 46,XY.
Voluntarily termination of gestation. Necropsy confirmed the ultrasonogram findings.
Second pregnancy: Ultrasonogram at 24 weeks revealed growth retardation, bilateral pyelic ectasia and moderate retrognathia. Parents decided to continue with pregnancy. 35 weeks: ultrasonogram showed growth retardation, prominent orbits and retrognathia; echocardiography revealed pericardial effusion without morphological cardiac alterations. 36 weeks: delivery was induced. Neonatal exploration: growth retardation, microcephaly, microretrognathia, dysplasic ears, festooned gums, craniosynostosis, hypospadias, cryptorchidia, pulmonary artery stenosis with persistence of the ductus, aracnodactyly in fingers and toes, bilateral glaucoma and generalized arthrogryposis. Karyotype: 46,XY. At age 3 months, the infant died with diabetes mellitus type I and hypertrophic cardiomyopathy. Necropsy report: weight 1.1 kg, cardiomegaly with myocardial hypertrophy and multiple septal infarcts, microcephaly, diffuse cortical anomalies, severe myelinization delay.
Diagnosis: The died patient was compatible with non-typical Seckel Sd. and Bowen Sd. The previous fetus was compatible only with Bowen Sd.
Conclusions: Because both Seckel and Bowen Sd. are infrequent entities, our belief is both cases correspond to Bowen Sd. Recurrence risk for these parents is 25% per pregnancy. Prenatal diagnosis is limited to ultrasonographic findings.

P0187. Child with del 11q23- ter - therapeutic problems

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Jacobsen syndrome is a rare cytogenetic abnormality, the literature reports about 30 children with this aberration. Main features of affected patients are: psychomotor development retardation, trigonocephaly, microcephaly, dysmorphy (low set ears, hypertelorism).
Here we present a boy with the diagnosis of Jacobsen syndrome. Reason for refferal was dysmorphy. During physical examination we found; low birth weight, microcephaly, psychomotor retardation, trigonocephaly, hypertelorism, wide nose with aplastic bridge, low set and dephormed ears, clinical features of laryngomalatia. Our cytogenetic examination revealed: 46, XY del 11q2- ter (G-banding).We decided to present the boy because of therapeutic problems, i.e; laryngomalatia, reccurent infections of lower respiratory tract, especialy pulmonary infections, renal malformations, reccurent infections of genitourunary tract. Severe mental retardation required neurological care and neurologic disturbances and delayed physical development were observed as well.
We want to underline the fact, that child with dysmorphic features requires cytogenetic examination and the care over child is a multidisciplinary problem.

P0188. Two new cases of the Clark-Baraitser syndrome

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The Clark-Baraitser syndrome of multiple congenital anomalies, tall stature, macrocephaly and mental retardation, has been in a limbo for many years. In the original report (Am J Med Genet 26: 13-15,1987), Clark and Baraitser suggested that their patients, two brothers and a carrier mother, may have the Atkin-Flaitz syndrome. In the subsequent description of affected cousins (Am J Med Genet 57:380-384,1995), Baraitser et al. favored the view that all of their patients had a condition distinct from the Atkin-Flaitz syndrome. We support the same view, by describing two brothers, who do not have the Atkin-Flaitz syndrome and strongly resemble Baraitser's patients. These boys are obese, macrocephalic, one of them excessively tall and both have a characteristic face with square forehead, prominent supraorbital ridges, bulbous tip of nose, short philtrum, gap between upper central incisors, large ears. Genitalia are normal. They also have big hands and feet and advanced bone age. They are moderately-to-severely retarded, with a quiet but stubborn personality. On brain MRI they have lateral ventricular dilatation, cortical and cerebellar vermis hypoplasia. The fragile X syndrome was ruled out by DNA testing and the Simpson-Golabi-Behmel syndrome can also be ruled out for lack of specific signs. The FG syndrome may be worth testing, once the gene has been identified. However, our prevailing view is that these cases confirm the existence of the Clark-Baraitser syndrome as a nosologically distinct entity that should be added to the list of X- linked mental retardation syndromes.

P0190. Kabuki syndrome: clinical data in 21 patients, literature review and guidelines for preventive management

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Kabuki syndrome (KS) was first described in 1981 independently by Niikawa et al. and Kuroki et al. Since than, over 250 reliable cases have been reported from many countries. Etiology of KS is still unknown.
In close collaboration with the Dutch network of Kabuki syndrome, 21 patients were studied. There were 7 males and 14 females, ranging from 2 years to 34 years. In addition, we reviewed the data of 260 KS patients (127M and 133F) in literature and compared them with those in our group. In this presentation we focus on the medical data and tables reviewing the data will be presented. Psychological - and language/speech data are reported separately.
In general, facial features are characteristic with long palpebral fissures, a thin upper lip and full lower lip with 'lip pits' without classical pits. Many patients have hypodontia with at least 2 upper incisors missing. Hands are small. The fingers show fetal fingertip pads. Mental retardation generally is moderate and the KS individuals have a positive personality.
We propose guidelines for preventive management. In early childhood the hypotonia, feeding problems and joint laxity pose major problems. The cleft palate needs surgery; recurrent ear infections and possible hearing loss are important issues. Growth and development should be closely monitored. Overweight can occur in early puberty.

P0191. Autosomal dominant ulnar/fibular ray defect: a possible new syndrome

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Postaxial oligodactyly with or without limb defects is most commonly an isolated anomaly. There are a few syndromes presenting with ulnar ray defects and postaxial finger malformation/reduction, however, these occure mostly sporadically. The highly penetrant ulnar-mammary syndrome includes postaxial ray defects, abnormalities of growth, delayed sexual development and mamillar and apocrin gland hypoplasia. We describe a three-generation family with variable expression of ulnar/fibular hypoplasia, ulnar ray defects and short stature. The proband had ulnar hypoplasia with missing IV-Vth fingers, fibular hypoplasia on the right, bilateral club feet, growth retardation, hypoplastic midface, ASD and hemangiomas. She had normal mamillary tissue and normal sweating. The mother had short stature, midfacial hypoplasia, hypoplastic ulna and hypoplasia of the carpal bones in the ulnar ray (brachydactyly type IV) on the right without other associated malformations. A maternal grandfather had mild unilateral fibular hypoplasia, and a maternal grandaunt had shortening of the IVth metacarpus of the left hand. Segregation studies did not confirm linkage to the locus (D12S79) of the Pallister UMS syndrome (MIM 181450). The patients may have a previously undescribed syndrome.

P0192. Cranioectodermal dysplasia: two new Egyptian cases with expansion of the phenotype.

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Cranioectodermal dysplasia is a rare syndrome characterized by craniofacial, ectodermal and skeletal dysplasia. We report two new cases, an eleven-year-old boy and a 2-year-old girl. The variability of clinical manifestations included dolicocephaly with or without sagittal suture synotosis, sparse hair, thin nails, brachydactyly, and advanced bone age. Varied dental anomalies consisted of microdontia with discrepancy of eruption and shedding, together with labial and jaws abnormalities. These cases are the first Egyptian cases of cranioectodermal dysplasia. Neuroimaging showed various changes in the form of periventricular hypodense areas and frontal atrophic changes. Fundus examination, electro-retinography, visual evoked potential, electro-cardiography, audiometry, IQ evaluation, abdominal sonar, kidney function tests, serum calcium, phosphorus and alkaline phosphatase were all normal. Advanced bone age in our two cases, which was not previously reported, expands the phenotype and indicates that cranioectodermal dysplasia is both a morphogenetic and maturation disorder.

P0193. Cotsirilos syndrome in twins from unaffected parents

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In 1987 Cotsirilos et al. described a family with 2 sibs and their mother presenting a Rubinstein-Taybi-like phenotype (MIM 180850). Autosomal or X-linked dominant inheritence was suggested.
We report on newborn male twins of healty unrelated parents with broad terminal phalanges of hallux, short stature, microcephaly, down slanting palpebral fissures, epicanthic folds, ptosis of eyelids, beaked profile of nose, low-set ears, high palate and a single palmar crease. A prominent metopic ridge was present, more prevailingly in one of the sibs,who also had pectus excavatum and hypospadias.
Early neurodevelopment was normal.
There were no cardiac or eye anomalies. The cranial ultrasound examination showed no brain malformation. X-ray examination of the skull revealed partial cranio-synostosis at the metopic suture and hypertrophy of bone and solid tissues of halluces.
Both children had a normal karyotype at 550-band resolution. FISH with DNA probes RT100 RT191, RT 166 (16q13.3:CREBBP) were normal.
Rubinstein-Taybi syndrome presents with broad hallux and typical facial features. The lack of typical cardiac and ophthalmolgic features and the presence of metopic ridge in our patients did not support this diagnosis. In craniosynostosis syndromes with broad hallux (Crouson, Apert) polydactyly/syndactyly is caracteristic, however it was excluded by the x-ray examination in our cases. br />The observed phenotype fits most probably into Cotsirilos syndrome and supports the primary observers' suggestion that it might be a distinct entity.

P0194. Byelorussian Down Syndrome Registry - rare cytogenetical abnormalities and clinical data characterization.

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We reviewed Down syndrome (DS) cases, registered in National Registry of Chromosomal Abnormalities during 1983-2000 years. Patients with mental retardation, congenital malformations and DS phenotype were studied by conventional cytogenetic method (GTG-banding, lymphocytes). Among 909 detected Down syndrome cases 834 patients with full trisomy 21 (included 18 cases with mosaicism) and 75 patients with DS due to de novo and inherited Robertsonian translocations (1 mosaic karyotype) were found. The following rare chromosomal abnormalities were observed: numerical aberrations - 48, XXY, +21 (2 cases); structural unbalance - 46,XX, inv dup (21) (two patients, in one case breakpoints were additionally investigated using FISH); complex rearrangements included 2 cases full trisomy 21 with balanced reciprocal translocations - 47,XX,t(11;21)(q21;q22)mat,+21; 47,XY, t(12;22)(p11.2;q13)pat,+21 and 6 cases pericentric inversions - 47,XY, inv(7)(p12q21.1)mat,+21 (1 case); 47,XX, inv(9),+21 (5 cases). Mosaic forms presented as numerical abnormalities - 47,XY,+21/48,XY,+21,+21 and 47,XY,+21/46,XX as structural 46,XX,t(21;21)/46,XX aberrations and complex rearrangements 48,XY,+21,+mar/46,XY. All individuals with DS including cases with rare cytogenetic variants and complex abnormalities demonstrated typical DS manifestations. In 2 mosaic cases the patients with low rate of the trisomic cells (8% trisomic metaphases) showed mild mental delay and less typical dysmorphic features. The cytogenetical and clinical data of DS will be discussed.

P0195. Pseudoachondroplastic dysplasia in a 4-years old boy.

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Pseudoachondroplasia (PSACH) is a spondyloepiphyseal dysplasia characterized by dysproportionate short stature with relatively long trunk, short bowed arms and legs and normal skull and face. On the basis of severity of radiographic findings Hall and Dorst (1969) classified PSACH into 4 types, 2 dominant (I, 177150 and III, 177170 MIM) and 2 recessive (II, 264150 and IV, 264160 MIM). In some cases gonadal mosaicism was proposed.

FP was the only child of young healthy nonconsanguineous parents of average height. He was born after 37 wk pregnancy with BW 3000 g (75th percentile) and BL 48 cm (75th percentile). His motor development was normal. Bowing of the legs and abnormal gait were present from 18 months. At 4 years of age he was 88 cm tall (3rd percentile) with good intelligence and showed normal skull, relatively long trunk, exaggerated lumbar lordosis, and asymmetric bowed legs. Radiographs showed widened metaphyses, small epiphyses of the long bones with delayed ossification, mild platyspondyly. Laboratory testing for metabolic diseases was normal. PSACH was diagnosed although the sub-type of the disorder was not verified.

Detection of PSACH subtypes in early childhood is difficult due to overlap the main clinical and radiological signs and limited pathological changes for young children. We will compare our data with those in the literature and discuss the differential criteria of the sub-types of PSACH.

P0196. Mutation analysis of the inhibin alpha gene in an Italian survey of women affected by ovarian failure

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Patients affected by premature ovarian failure (POF) (n=157), early menopause (EM) (n=36) and primary amenorrhoea (n=12) were analysed for the missense mutation (769G→A transition) in the exon 2 of the inhibin alpha gene (INHa). The incidence of the mutation was statistically significant within both the POF (sporadic and familial) (7/157, 4.5%) (Fisher's exact test, P= 0.030) and primary amenohrroea (3/12, 25%) (Fisher's exact test, P=9.6X10-4) patients, in regard to the control group (0/100), comprising women who experienced physiological menopause. No mutation was found in the group of EM patients. Furthermore, the likelihood of finding the mutation was statistically different for familial (5/65; 7.7%) (Fisher's exact test, P=8.6X10-3) and sporadic (2/95; 2%) (Fisher's exact test, P=0.23) POF conditions. Moreover, the analysis of pedigrees showing the running of both the 769G→A mutation and POF strengthens the concept of the disease heterogeneity, since the POF phenotype was not always associated with the mutation. During this work, we also evidenced the prevalence in the POF patients (80.3%) in regard to the control group (66.7%) of the C allele of a SNP, located in the 5'UTR of the INHa gene. Although these data tend to indicate that the INHa gene can be considered a candidate gene for premature menopause, the assessment of its true diagnostic value requires further investigations.

P0197. Meier-Gorlin syndrome: New clinical findings and exclusion of BMP5 as a causative gene

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Meier-Gorlin syndrome (MGS) or ear, patella, short stature syndrome (MIM 224690) is a rare autosomal recessive disorder, characterized by microtia, patellar a-/hypoplasia, and growth retardation. The most serious aspects are feeding problems and recurrent respiratory infections in early infancy. Here, we present the results of growth-, neuromuscular-, and immunological investigation in the largest cohort of MGS patients (8) described in literature. Endocrinological investigations showed normal IGF and growth hormone test results in most patients. Growth hormone therapy resulted in minimal/no effect in all four cases that received treatment. In two sporadic patients available for neuromuscular examination, proximal weakness and a-/hypotrophic ventral muscle groups of the upper and lower extremities was found. Light microscopy of quadriceps muscle biopsies revealed muscle fibre hypoplasia in one, but normal appearance in the other patient. Immunological studies showed decreased numbers of cytotoxic memory T-cells (CD8+ /CD45RO+) in the three examined infants.

Based on the striking similarities between MGS and the murine short ear phenotype caused by homozygous mutations in the BMP5 gene, Lacombe et al. [Ann Genet, 1994;37:184-191] proposed BMP5 as candidate gene for MGS. Recently, the BMP5 gene was sequenced in one MGS patient, and no mutations were detected [Cohen et al., Am J Med Genet, 2002;107:48-51]. Simultaneously, we excluded the BMP5 locus as candidate region by linkage analysis in two consanguineous families, including one family with three affected sibs. Homozygosity mapping in consanguineous MGS families is presently being performed as a first step towards the molecular identification of the gene responsible for MGS.

P0198. Alstrom syndrome - the overlooked syndrome? Case report and review. T.Datkhaeva, E. Elias and E. Sujansky. Division of Genetics and Metabolism, University of Colorado School of Medicine, the Children Hospital, Denver, UCHSC.

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Severe early-onset inherited cone dysfunction, secondary rod degeneration and obesity are the hallmarks of Alstrom syndrome, a very rare autosomal recessive disorder.

We present a 2 ½ year old male first seen in ophthalmology at age 8 months with nystagmus, retinal degeneration and bilateral high myopia. Other than repeated otitis media, he has been healthy. He has shown normal cognitive development. The patient's father and two paternal second cousins have retinal abnormalities.
Physical exam at age 10 months disclosed a weight of 50th% for age 2-years. Mildly dysmorphic features included telecanthus, narrow and high palate and equinovarus. Alstrom syndrome was suspected. Brain MRI, hearing tests and renal ultrasound were normal. His karyotype was normal (46,XY). When reviewed at 27 and 30 months he wad found to have progressive visual loss, hypertriglyceridemia and progressive obesity, despite caloric restriction. DNA-testing for Alstrom syndrome mutations on chromosome 2 is pending.
The differential diagnosis of cone-rod dystrophy presenting in early infancy is broad, but when associated with early obesity should suggest the diagnosis of Alstrom Syndrome. Sensorineural deafness, cardiomyopathy, hyperlipidemia, and diabetes may not present until later in childhood. In contrast to patients with Bardet-Biedl syndrome, patients with Alstrom syndrome display normal cognitive development, and normal digits. It is important that clinicians and ophthalmologists consider Alstrom syndrome in the differential diagnosis of retinal dysfunction. DNA testing is now available to confirm the diagnosis, and allow optimal subspecialty care for these complex patients.

P0199. Microsatellite mapping and screening of a candidate gene, TRPC5 (Transient Receptor Potential Channel) in a second family with Arts syndrome

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Arts syndrome was first described in a Dutch family with X-linked ataxia, muscle weakness in response to infections, deafness, loss of vision in early childhood and a fatal course. We describe a family with two affected brothers and a maternal uncle who died at 2 years of age of a "muscular dystrophy" without having developed any speech. The two brothers have profound sensorineural deafness, peripheral neuropathy with generalised muscle weakness and, in the older boy, symptomatic optic atrophy. They are predisposed to severe muscle weakness during recurrent infections which has led to mechanical ventilation on a number of occasions.
To localise the region for this syndrome, an exclusion mapping approach was taken, using 33 microsatellite markers covering the X chromosome. DNA was available from the affected brothers, their normal sister, parents, maternal grandparents and an unaffected maternal uncle. Two candidate regions were identified: Xq23 between DXS 1106 and DXS 8064 (~13cM), and Xq27 between DXS 1227 and DXS 8091 (~21.4cM). The candidate region Xq21.33-q24 was identified in the original family, so the Xq23 region was deemed most likely. Databases were searched for likely candidate genes. TRPC5 was selected as the most likely because of its involvement in Ca2 flux, and its expression in mammalian brain and Drosophila eye. Screening of this gene is in progress. To date one A>G change has been identified at 919-42 (intron 1) in the affected boys, their normal father and maternal grandfather. It is therefore likely to be a common polymorphism.

P0200. Absent sacrum in terminal deletion of the long arm of chromosome 7:a further case

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Small deletions of chromosome 7qter are rarely reported. The literature contains 7 cases with deletions distal to 7q35. In conventional cytogenetics these deletions may be overlooked, so that clinical characteristics have to be elaborated to further delineate a specific syndrome.
Case report: The girl was born as the second child to a 22 years old mother and a 28 years old father. She was born at 39 weeks of gestation. She had low birth weight with 2625g (5th centile), length: 47cm(10th centile), head circumference:30,8cm(<10th centile). The cry was weak and she had severe problems with sucking, so that gavage feeding was necessary. The most striking features were microcephaly with a narrow forhead and large dysplastic ears with a wide exernal meatus. The palpebral fissures slanted up. The nose was short with a bulbous tip, the philtrum was simple, the maxilla hypoplastic. The muscle tone was generally reduced. The EEG was abnormal. X-ray showed thin ribs and an vertically absent sacrum, which could not be suspected clinically.Sonographic investigations revealed a tethered cord with a very low lying conus medullaris inserting in a small lipoma. Hearing was severely impared. Standard chromosomal analysis was normal, but subtelomeric analysisis using a comercial multitelomere FISH.kit(Cytocell) revealed the absent signal on 7qter. The parents chromosomes will be studied.
We stress that children with micrcephaly and absent sacrum should be looked for a cryptic deletion on 7q.

P0201. Gorlin Syndrome in a patient with deletion of the distal part of chromosome 9q and fine mapping of the break points with Fluorescence In Situ Hybridization (FISH).

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We present a 20 year-old male with many nevi of the skin and three basal-cell carcinomas who was referred by a dermatologist for further investigation. Previously a chromosome analysis was carried out showing the karyotype 46,XY,del (9)(q21.3;q31) de novo. *PTCH* located at 9q22.3 was shown by FISH to be deleted confirming the diagnosis of Nevoid Basal Cell Carcinoma Syndrome or Gorlin Syndrome. In addition he has developmental delay, dental and cardiac malformation and dysmorphic features, probably caused by loss of chromosomal material around *PTCH*. Further characterization of the deletion showed the proximal breakpoint at 9q22.2 and the distal breakpoint distal to 9q31.2. Further fine mapping will be presented.

P0202. A family with Seathre Chotzen syndrome and mutations in the TWIST and FGFR2 genes

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Most cases with Saethre-Chotzen syndrome (SCS), a relatively frequent autosomal-dominant craniosynostosis syndrome, are due to mutations in the TWIST gene. Other SCS patients carry the missense mutation P250R in the FGFR3 gene, leading to a similar phenotype. The classical SCS phenotype includes brachycephaly or acrocephaly and partial cutaneous syndactyly of hands and feet.
The phenotypic expression of SCS is highly variable also within families. These variability is very likely due to an influence of modifying genes. Under the assumption that special alleles of the FGFR1, 2 or 3 genes may be such modifiers, we investigated several members of a German SCS family. Two members of this family, a 6 months old boy and his father, displayed the classical SCS phenotype. With the exception of partial cutaneous syndactyly, no further symptoms were present in the boys paternal uncle, grandmother and grand-grandmother.
Both the boy and his father carried two mutations: D161X in the TWIST gene and S252L in the FGFR2 gene. D161X had previously been described in a SCS patient, and S252L was associated with a mild Crouzon phenotype. No other family member carried D161X. However, S252L was present in all family members with syndactyly. We conclude that D161X arose de novo, leading to the classical SCS phenotype, while S252L was associated with cutaneous syndactyly only and spread from the paternal grandmother over the whole family. To the best of our knowledge, this is the first family in which both a TWIST and a FGFR2 mutation were present in SCS patients.

P0203. Association of PAI-1 gene 4G/5G genotype with Coronary Artery Disease

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The relation of PAI-1 gene promoter 4G/5G genotype and the risk of developing Coronary Artery Disease (CAD) is still controversial. The objective of this study is to evaluate the role of 4G/5G genotype on development of CAD. Distributions of 4G/5G genotype were studied in consecutive CAD patients with Myocardial Infarction (MI, n=158), Stable Angina Pectoris (SAP, n=124) and in 282 unrelated healthy controls. 1- The frequencies of both 4G/4G and 4G/5G genotypes were 33% and 48% respectively in CAD patients with MI and 26% and 40% respectively in controls, the differences in the frequencies of both genotypes were statistically significant (OR: 2.3 and 2.2; 95% CI 1.3-4.0 and 1.3-3.6 respectively). 2- There was statistically significant differences in 4G/5G genotype between CAD patients with SAP (57%) and control (40%) (OR: 1.9; 95% CI 1.2-3.2). 3- Comparison of the frequencies of 4G/4G genotype in patients with MI (33%) and SAP (17%) was statistically significant (OR: 2.6; 95% CI 1.3-5.4). Hyperlipidemia appeared to be an additional risk factor for development of MI in patients with 4G/4G genotype (OR: 3.0; 95%CI 1.5-6.2). Although smoking is an independent risk factor (OR: 1.7; 95% CI: 1.1-2.8), it did not effect the risk of developing MI associated with 4G/4G genotype. The results of the study suggested that 4G/4G and 4G/5G genotypes may be associated with an increased risk of developing MI while presence of only 4G/5G genotype seemed to be related to the risk of developing SAP. This study was supported by Hacettepe University research grant (9902101003).

P0204. Coffin-Siris syndrome in a girl with a 15qter deletion encompassing the IGF-1 receptor gene.

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We report on a 11.5-year-old girl with features of multiple congenital anomalies/mental retardation overlapping those of Coffin-Siris syndrome (MIM 135900) and a deletion on 15 qter, not seen by standard karyotype, but only by multiFISH subtelomeric assay. Weight, length and OFC were all below the third centile at birth. Impaired sucking and swallowing were referred up to 8 months. Developmental delay was noted since the very beginning. She shows now severe mental retardation, dwarfism (height 102 cm), microcephaly, large mouth with flat philtrum, unilateral palpebral ptosis, hirsutism on the lombosacral area. Hands and feet are very small with shortening of fifth fingers and fourth and fifth toes, and nail hypoplasia of fifth fingers and of second, third, fourth and fifth toes. X-ray examination reveals very short terminal phalanx of the fifth fingers and toes, double epiphisis on second, third, fourth and fifth methacarpal bone and short middle phalanx of fifth finger. The bone age is severely retarded (6.1 years). Growth hormone, IGF-1, IGFBP-3, thyroid function are normal. As additional features the girl has multicistic kidney with mild renal failure. Previous reports by McPherson et al. (1997) and McGhee et al. (2000) assigned a locus for the Coffin-Siris syndrome to the 7q32-q34 region. Our report may raise the chance of a second locus for such syndrome in the 15qter region. We conclude highlighting the presence in the 15q25-q26 region of the IGF-1 receptor gene, which may be a clue for the etiology of the phenotype and possibly for the Coffin-Siris syndrome.

P0205. A novel approach for determining the parental origin of GNAS1 mutations in sporadic patients with Albright's Hereditary Osteodystrophy using overlapping imprinted transcripts: Clinical application for predicting endocrine phenotype.

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Albright's Hereditary Osteodystrophy (AHO) results from heterozygous deactivating mutations in the GNAS1 gene which encodes the alpha subunit of the adenylyl cyclase stimulatory G-protein, Gs. Associated clinical features include short stature, obesity, brachymetaphalangia, ectopic ossifications and learning disability. Inheritance is autosomal dominant but modified by genomic imprinting. Maternal transmission is associated with the endocrine features of pseudohypoparathyroidism (PHPIa) whereas paternal transmission is usually not (pseudo-pseudohypoparathyroidism, PPHP).
GNAS1 comprises 13 exons and the imprinting appears to be tissue specific. However, 3 discrete upstream exons (NESP55, XLAS, exon 1A) which are spliced to exons 2-13 of GNAS are imprinted in all tissues tested. Expression is exclusively maternal for NESP55 and paternal for XLAS and 1A.
We have screened for sequence changes in all the exons and splice junctions of GNAS1 in a cohort of patients with AHO and found a variety of mutations. We have confirmed that imprinting of NESP55 and exon 1A is conserved in lymphoblastoid cell lines. In AHO patients with mutations in exons 2 - 13 of GNAS from whom lymphoblastoid cell-lines were available, we have tested directly for the mutation in the NESP55 and 1A cDNA transcripts. In 3 sporadic patients with PPHP we have confirmed the mutation is present in the 1A transcript, but not NESP55 indicating paternal origin. In 6 patients with AHO and PHP Ia we have confirmed the converse indicating maternal origin. With the discontinuation of PTH for stimulation testing, the clinical application for predicting the endocrine phenotype in AHO patients will be discussed.

P0206. A case of Zimmermann-Laband syndrome with a chromosomal translocation t(3;8)(p13;q24.1)

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Zimmermann-Laband syndrome (ZLS) is a rare autosomal dominant disease characterized by gingival hyperplasia or fibromatosis, hypoplasia of the finger- and toenails, hypoplastic changes in the terminal phalanges of fingers and toes, coarse facial features, hepatosplenomegaly, inconsistent mental retardation (Pfeiffer et al, 1992). All reported cases have normal karyotype. Molecular basis of this condition remains unknown.
Here we report on an apparently balanced chromosomal aberration, t(3;8)(p13;q24.1) in a mother and her daughter, both with typical ZLS features characterized by gingival hyperplasia, finger- and toenails hypo- and aplasia and course facial features, including large nose, thickened lips and flashy ears. None of the closely related family members were affected with ZLS, nor had a chromosomal aberration. It is suggested that the causative gene for ZLS is located at the translocation breakpoint(s).

P0207. Segregation of a t(1:3)(q42.3;p25) Translocation Resulting in Different Recombinant Chromosomes in Multiple Family Members

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A subtle familial balanced translocation involving the terminal regions of 1q and 3p was identified in a large family by high-resolution karyotype analysis and confirmed by (FISH) analysis.
In this family, segregation of a balanced t(1:3)(q42.3;p25) chromosome translocation in two phenotypically normal sisters led to two types of viable unbalanced complements which corresponded to two distinct phenotypes in affected individuals. The proband had inherited the derivative chromosome 3 resulting in partial trisomy of chromosome 1q and partial monosomy of chromosome 3p. A paternal uncle and cousin had the reciprocal rearrangement with a derivative of chromosome 1 resulting in partial monosomy for chromosome 1q and partial trisomy for chromosome 3p.
While profound mental and physical retardation, poor survival, congenital heart defects, and neurologic abnormities were characteristic for both rearrangements, facial dysmorphism was quite distinct for each recombinant. Individuals who had the derivative chromosome 3 had a long face, wide eyebrows, small palpebral fissures, hypertelorism, prominent glabella, a large tip of the nose, long philtrum with thin upper lip, and low set-ears with prominent helices. In contrast, family members with the derivative chromosome 1 had a tall forehead with bifrontal narrowing, full and large cheeks, and large simple ears.
In this kindred, the ratio of normal to abnormal individuals born to balanced carriers is believed to be 1:1.5. This suggests that the recurrence risk for carriers is at least as great as 50%.

P0208. A Variant of Whistling Face Syndrome or A New Syndrome?: A Case Report

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We present a 4-hour old male infant born at term to healthy parents who related as first cousins, with spontaneous vaginal delivery

after an uncomplicated pregnancy. His weight, length and head circumference were 2250 g, 43 cm and 33 cm, respectively. The boy showed immobile face, hypertelorism, blepharophimosis, antimongoloid eye slant, bulging cheeks, small nose, small mouth, symmetric clenched fingers, camptodactyly, ulnar deviation of the hands. These clinical findings suggested ''Whistling Face Syndrome''. In addition, intrauterine growth retardation, microcephaly, microphthalmos, micrognathia, bilateral incomplet choanal atresia, laryngomalasia, low and malforme ears, short neck, wide spaced nipples, pelvicaliectasia, uretheral dilatation, absence of one costa, sandal gap and weak primitive reflexes were determined. Chromosomal analysis was normal (46, XY). We suggested that this patient is a variant of Whistling Face Syndrome or a new syndrome

P0209. Blepharophimosis to craniostenosis: the human and murine phenotypic spectrum related to TWIST haploinsufficiency

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Introduction: The TWIST gene, a bHLH transcription factor, is mutated in patients with Saethre-Chotzen syndrome (SCS). Twist null/+ mice have been found to reproduce the main clinical features of SCS.
The aim of this work is the analysis of the phenotypic spectrum related to haploinsufficiency in the TWIST gene through the study of a large Indian family and the observation of the murine phenotype.
Material and Methods: The TWIST gene was a candidate gene in a large Indian family presenting with an autosomal dominant phenotype initially classified as Blepharophimosis-Epicanthus Inversus Syndrome (BPES) linked to 7p21.
The eyelid phenotype of twist null/+ mice bred on different genetic backgrounds was observed.
Results: A new nonsense mutation, at codon 82, was detected for 16 members of the family (excluding the 7p21 locus as the second BPES locus as initially suggested).
The clinical reappraisal of these 16 individuals carrying the mutation identified a complete SCS phenotype for only 4 patients (25%). 75% of the patients did not show craniostenosis, 25% had a phenotype considered to be normal and 75% of the patients had eyelid anomalies. Isolated eyelid anomalies, without any cranial anomalies, were also observed on Twist null/+ mice bred on a mixed background.
Conclusions: The clinical analysis of the Indian family confirms the wide variability of the phenotype related to TWIST haploinsufficiency ranging form a normal phenotype, an isolated eyelid malformation to severe craniostenosis. In parallel, the wide phenotypic variability was also observed in twist-null/+ mice bred on different genetic backgrounds.

P0210. Dup(3)(q26>qter) & del(3)(pter>p25) Due To Paternal Pericentric Inv(3) But With Phenotypic Abnormalities Not Consistent With Both Well-known Clinical Syndromes - A Case Report

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Cytogenetic studies remain the cornerstone in defining mental retardation syndromes associated with dysmorphic features. Chromosome 3 structural aberrations to a considerable extent result from unbalanced recombinations. We report on a 5 year old boy who by detailed cytogenetic procedures was diagnosed as having a distal deletion 3p as well as a duplication (3)(q26>qter) due to a large pericentric inv(3) inherited from his father. In addition to conventional karyotyping, final diagnosis was achieved using multiprobe telomer testing (Cytocell) and region-specific FISH probes. Theoretically our patient should express both the partial dup (3)(q26>qter) and the deletion (3)(p25>pter) syndromes. In contrast we noted a mild but apparent dysmorphic pattern with distinct features - square face, temporal indentation, broad eyebrows, full cheeks, bulbous nose, and full lips - suggestive of a mitigated partial dup 3p syndrome. No major malformation was observed. Causal parental pericentric inversion was reported in a number of cases with similar breakpoints, but the children were mostly severely affected with short survival. This report should draw attention to some interesting details: (i) Loss of the most distal region of 3p may be noticed without phenotypic effect (Knight, J Med Genet 1995).(ii) Prediction of the phenotype seems difficult even in well-described conditions like 3qter-syndrome (iii) In a given pericentric inversion, survival would be more probable in great duplication plus a small subtelomeric deletion than vice versa.

P0211. Trigonocephaly and associated urinary anomalies in mother and son

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A mother and her son are described with neonatal trigonocephaly, multiple suture synostosis; shallow orbits; unusual nose; deviation of the terminal phalanges of fingers 1, 2 and 5; and broad toes which radiologically may show duplication of the terminal phalanx. Untreated, the condition leads to a disfiguring oxycephaly with hypotelorism.
This appears to be the first documented instance of autosomal dominant trigonocephaly.
The importance of the minor anomalies in its recognition and its good prognosis are emphasized.

P0212. Clinical study of a case of Chondrodysplasia Punctata Conrady-Hunermann type

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We are presenting a case of chondrodysplasia punctata Conradi-Hunermann type in order to illustrate this rare entity and discuss the importance of different features for the diagnosis, differential diagnosis and genetic counselling.
Our proband is a male infant, first child of a healthy, young, unrelated couple, first examined in the Maternity Hospital (4 days old). Clinical features: mild dysproportionate short stature (short limbs with contractures); dysmorphic face (coarse face, frontal bossing, flat, broad nose, macrostoma, abnormal ears); ichthiosiform erithroderma with curled, linear distribution, keratotic papules (fingers and toes); club foot. 7 months later physical examination showed: dysproportionate short stature (short limbs, rhizomelic segment mainly affected), the same dysmorphic face, depigmented curled linear areas of skin (trunk, abdomen and limbs), follicular atrophoderma and bilateral club foot (ortopedically treated). X-ray investigation: stippled calcifications (proximal humeral epiphyse and sacral area), short humeral and femoral bone and delayed ossification of the femoral head. Karyotype: 46,XY. We have established the diagnosis of chondrodysplasia punctata Conradi-Hunermann type. Detailed positive diagnosis, differential diagnosis (of dysproportionate short stature and linear ichthiosiform erithroderma) and problems of the genetic counselling will be provided.
In conclusion, we present a case of chondrodysplasia punctata Conradi-Hunermann type (AD) to discuss different diagnostic aspects.

P0213. Molecular Analysis In Patients With Mayer-Rokitansky-Kuster-Hauser Syndrome

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Müllerian agenesis, also known as Mayer-Rokitansky-Kuster-Hauser syndrome (MRKHS) is the second most common cause of primary amenorrhea. The syndrome involves the absence of the fallopian tubes, the uterus, and the upper third of the vagina in otherwise normally developed females. To date the etiology has not been elucidated and the search for molecular variants in these patients has been focused to genes involved in the Müllerian regression

system. We present the results of molecular studies in the AMH, AMH receptor, and GALT (galactose-1-phosphate uridyl transferase)genes in a group of 12 Mexican patients with MRKHS. Methods: DNA extraction from blood leukocytes, PCR amplification of all exons and all exon/intron junctions of the AMH and AMHR genes, and PCR amplification of exon 10 of GALT. Results: No mutations were found in any of the patients in the AMH or AMHR. However, we identified 5 new polymorphisms: two in intron 6 of the AMHR gene, 1 in exon 1 of the AMH gene, and two in exon 5 of the AMH gene. All three polymorphisms in the AMH gene change the encoded aminoacid. When compared with 30 control alleles AMH exon 1 polymorphism showed a preliminar association with the disease. The GALT N314D allele, previously associated with the disease, was observed only in one of our patients. These results demonstrate that mutations at the AMH or AMHR genes are not a common cause of MRKHS. Polymorphisms at the AMH or AMHR genes may contribute to develop the phenotype.

P0214. Maternal Heterodisomy for chromosome 14, and 13/14 Robertsonian Translocation, in a female with normal mental development, short stature and dysmorphic features

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Maternal Uniparental Disomy for chromosome 14 was reported in the literature in about ten subjects, after the first description by Temple in 1991. A distinct maternal UPD(14) phenotype is emerging from the published data, but the clinical spectrum is only partially homogeneous.
We report on a new case of maternal heterodisomy for chromosome 14 in a thirty years old female, referred to our Centre, with her husband, for genetic counseling aimed to procreation.
Physical examination of the proposita showed a completely normal mental development, with short stature recalling hypochondroplasia, but associated with unrelated dysmorphic features.
Cytogenetic analysis showed a balanced, *de novo*, Robertsonian translocation 45,XX,der(13;14). Based on the cytogenetic results, we performed the molecular analysis for UPD (14), by using a battery of microsatellites markers in the lymphocites DNA of the proposita and her parents.
A maternal heterodisomy for chromosome 14 was demonstrated by several informative markers, derived from different regions of short and long arms: we confirmed these results by means of a paternity testing with normal biparental contribution. FISH analysis was also completed. From the clinical point of view, of special interest is the long and well documented natural history of this patient, that adds new elements to the clinical spectrum of this rare disorder. We also report data on the skeletal morphology and development, and clinical phenotype at different ages.

P0215. *De novo* partial duplication of chromosome 15, resulting from an unbalanced translocation of an extra segment 15p13-q22 on the short arm of a chromosome 8, in a child with severe and global development delay

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The proposita is a newborn female of Albanian origin, referred at age 23 days, for severe prenatal and postnatal growth, “anaemia” and dysmorphic features.
Cytogenetic analysis showed, in all metaphases of the proposita, an abnormal short arm of chromosome 8, with the presence of extra material of unknown origin, translocated on the 8p23 band. The chromosomes of the parents were normal.
Molecular cytogenetic analysis was performed to characterise this chromosomal rearrangement. FISH, using a chromosome 8 and chromosome 15 painting probes, demonstrated an apparently pure, partial Trisomy of chromosome 15. Molecular analysis, using microsatellite DNA markers mapping to the short and long arms of chromosome 15, showed that the duplication was of maternal origin.
Proximal Trisomy 15q is a rare disorder and the clinical spectrum is not well characterised. Microcephaly and cardiac malformation, described a few times, are also present in this new patient.
Mental retardation is to be expected: it is not yet well assessable in the proposita.
A bone marrow dysplasia is documented, and could represent a major aspect of the karyotype/phenotype correlation, and of the prognostic problems for this child.

P0216. Identification of a TP63 gene R298Q mutation in a family with EEC/ADULT syndrome phenotype.

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G.N., a boy, presented at birth with: absence of right hand middle finger, skin syndactyly of feet, polydactyly of right foot and bilateral obstruction of tear ducts. At physical examination, at 4 years of age, we noted an albinism-like appearance with very lightly pigmented hair and skin, dryness of skin and high photosensitivity, brittle and dysplastic nails with pitting, hypoplasic teeth and hypoplastic nipples. He suffers from chronic conjunctivitis. His mother, C.R. (who had never been investigated before), presented: dry and lightly pigmented skin with high photosensitivity and “dermatitis-like” lesions of face and hands, brittle and dysplastic nails with pitting and dysplastic teeth with absence of a permanent canine tooth. She now has dark blonde hair but she had lighter hair in childhood. She also suffers from chronic conjunctivitis. The clinical phenotype we observed in G.N. suggested differential diagnosis between EEC (ectrodactily, ectodermal dysplasia, cleft lip/palate) and ADULT (acro-dermato-ungual-lacrimal-tooth) syndromes. According to autosomal dominant inheritance and variable expressivity, recognized for both syndromes, we thought C.R. could be mildly affected be the same disorder of his son. TP63 gene (3q27) mutations have recently been identified in patients with either EEC or ADULT syndrome. Mutation analysis in this gene revealed a heterozygous R298Q mutation in both the patient and his mother. The same mutation had been previously identified in a large ADULT syndrome family. The proband and his mother, and other family members who are likely to be equally affected, will be further investigated to better define clinical diagnosis and genotype-phenotype correlation.

P0217. Report of a Rare Case of Fibula Aplasia and Complex Brachydactyly

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This fact that there is not fibula in the human body is one of the clinical signs of several rare syndromes which is often autosomal recessive. The case of study is a four-year-old girl in whom this fact that there is not the bi-directional fibula has been observed. No mental retardation is observed in this child.
In radiographic studies no fibula as well as phalanges and metacarpus are visible in the child’s right hand. The tibia bones are very short. The family pedigree of this child shows that her parents had family marriage with the relative of aunt’s son-aunt’s daughter (F=1/16). But, no other similar case wase observed in the family. This case was compared with fibula aplasia and complex brachydactyly (MIM: 228900). As a result, though there were many similarities, some differences were observed in this case.

P0218. Detection of 5FU-toxicity-related variants in the DPYD gene by denaturing HPLC.

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A decreased dihydropyrimidine dehydrogenase (DPD) activity has been described in cancer patients with intolerance to the fluoropyrimidine anticancer drug 5-fluoro-uracil (5FU). So far, 17 inherited mutations probably related to this impaired enzyme function were found in the DPYD gene. We performed a research-based population study to unravel new mutations and to get sufficient data to determine genotype-phenotype relations in the DPD deficiency syndrome. The entire coding region and the exon/intron boundaries of the DPYD gene were screened by denaturing HPLC (DHPLC). DPD

protein levels were quantitatively analyzed by ELISA and DPD mRNA levels were determined by using a DPD mRNA Quantification Kit. Several polymorphisms and intron variants were frequently detected in 160 German individuals. The 85T>C mutation as well as the 1601G>A substitution, both controversially discussed as mutations with relation to 5FU-toxicity in the literature, could be detected in various individuals with normal DPD protein level and additionally in a patient showing toxic reactions under CMF chemotherapy. Finally, the splice mutation IVS14+1G>A and the frameshift mutation 296delTCAT which result in truncated proteins devoid of activity were found in the heterozygous state in our population study. The data are correlated with DPD protein- and DPD mRNA expression level. The rapid and sensitive screening of cancer patients for mutations associated with 5FU-intolerance might be useful prior to the onset of chemotherapy because the risk for 5FU-related toxicity is not entirely rare. We show that the DHPLC technology could be a reliable tool for mutation detection in pharmacogenetics.

P0219. A third case of progressive macrocephaly with dilated Virchow-Robin spaces on MRI

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We report the case of a boy referred for a quickly progressive macrocephaly. He was the first child of young non-consanguineous and healthy parents. The family history was unremarkable.
Clinical evaluation revealed at 6 months of age a head circumference (HC) at +5 SD whereas the HC was +2 SD at birth. Neurologic exam was normal except a minor axial hypotonia. Neither associated dysmorphic features nor delayed motor development were noted at this time. No signs of intracranial pressure were present. Magnetic resonance images showed prominent Virchow-Robin spaces in occipital regions without increased size of the ventricles or involvement of the cerebral white matter. This pattern is usually a sign of storage disease but after investigations, there was no argument in favor of lysosomal disorder or muco/oligosaccharidosis. We also excluded a mitochondrial disorder.
The present case appears similar to those reported by Artigas et al. in 1999, which exhibited a progressive macrocephaly with an onset in the first year of life, dilated Virchow-Robin (DVR) spaces within the white matter of the cerebral convexities and normal neurodevelopmental status. Our reported case agrees with the hypothesis that macrocephaly with DVR can be considered as a new entity. Follow-up of our very young patient in the future will be necessary to confirm the good neurological prognosis of this entity.

P0220. Molecular genetic delineation of *de novo* 7p11-p14 deletions in Greig syndrome

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We have identified six children with a *de novo* deletion involving the chromosomal band 7p13 associated with Greig cephalopolysyndactyly syndrome (GCPS) and various other clinical features. Here we want to focus on the delineation of the phenotype in all patients in particular on those symptoms, that are not typically related to GCPS like a moderate psychomotor retardation, seizures, muscle fibre anomalies, cardiac anomalies, hyperglycaemia or hirsutism. We studied genotype-phenotype correlation in our patients, by using the combination of classic cytogenetics, FISH, and the analysis of polymorphic DNA markers. All deletion breakpoints were precisely mapped and based on these results in combination with genomic sequence data available by now, we were able to identify several candidate genes mapped to the deleted chromosomal segments.
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P0221. Ohdo blepharophimosis syndrome: report of two new unrelated cases and review of literature

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Ohdo blepharophimosis syndrome (OBS) is a rare condition first reported by Ohdo et al. (1986). The exact aetiology of this syndrome has not been established yet. Here we report two new unrelated cases of OBS and review the literature.
Both our patients had moderate developmental delay, peculiar facies with blepharophimosis, ptosis, broad nasal bridge, flat nasal tip, flat philtrum, small mouth, and low-set dysplastic ears, and muscular hypotonia in neonatal period.
Case 1 was born prematurely on 35th week of pregnancy with proportionate failure to thrive (-3.0 SD). Soon after the birth the heart defect was diagnosed (supravalvular aortic and pulmonary stenosis). Clinical evaluation at age 4.5 years showed proportionate failure to thrive (-3.0 SD). Her teeth were peg-like, irregular and with dysplastic enamel. Clinodactyly of 5th fingers was also observed.
Case 2 was born at term with low birth weight (-3.0 SD). She had probably some alcohol exposure during the pregnancy. Our examination at age 7 years revealed failure to thrive (-2.5 SD), microcephaly (-4.0 SD), high palate, dental hypoplasia, hypertrophic gingivitis, bilateral cross-bite, anterior open bite, ear canal stenosis, and café au lait spot.
Overall 16 cases have been described until now. Our patients show similar features to the previously reported patients with OBS. All previously described had blepharophimosis and developmental delay. The second most common finding was dental abnormalities. Features, occurred in two-third of the cases, were ptosis, broad and/or flat nasal bridge, distinctive nasal tip, dysmorphic and/or small ears, failure to thrive and muscular hypotonia.

P0222. CATCH phenotype: 6 cases from Estonia, three confirmed by FISH

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The CATCH phenotype (Cardiac abnormalities, Abnormal face, Thymic hypoplasia, Cleft palate, Hypocalcemia) comprises developmental defects of the 3rd and 4th pharyngeal pouches and other areas. Frequency is 1 in 4000 live births. Clinical signs are highly variable. About 90% of affecteds demonstrate the typical 22q11 deletion, which in 10-20% of cases may be inherited. - We report on 6 unrelated infants, age newborn to one year, who showed clinical signs of the CATCH phenotype and were seen at Tallinn Childrens Hospital. Complications during pregnancy (drug,alcohol abuse, threatened abortion) occurred in 4 families. All subjects demonstrated dysmorphic signs. Five had a congenital heart defect (truncus arteriosus communis [3 cases]; tetralogy of Fallot; combined ASD and VSD). In one child, thymus aplasia was observed during cardiac surgery. Two out of three investigated probands showed reduced numbers of T and B lymphocytes. Hypocalcemia was observed in 5 children. - FISH studies were performed in 4 children using a set of 4 DNA probes, (i) BAC 201c11 that detects the common large and the proximal 22q11 deletion (area, HIRA/D22S553/D22S609), (ii) BAC 219g6 detecting the common large but not the proximal 22q11 deletion (area, HCF2), (iii) BAC 384d8 (ARSA, 22q13, reference probe) and (iv) PAC 323N1 that detects the DGS2 area on chromosome 10p14. Three children demonstrated the typical common large 22q11 deletion.For diagnostic purposes and genetic counselling, we now aim to maintain a stringent policy of studying all our patients with probably CATCH phenotype for the presence of the 22q11 deletion.

P0223. Juvenile Hyalin Fibromatosis in three sibs from a consanguineous family: Clinical, histopathological and imminohistochemical findings

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Juvenile hyalin fibromatosis (JHF; Murray-Puretic-Drescher Syndome; OMIM #228600) is a rare autosomal recessive disorder characterized by progressive tumors in the skin and scalp, flexion

contractures of joints. We have observed a consanguineous family from Urfa, Turkey with three affected (8 and 3 years old boys and 1 year old girl) and two normal sibs. All affected sibs were normal at birth. Flexion contractures and multiple subcutaneous fibrous tumors initiated in a range in between two months to 4 years of life. The hyalin tumors were distributed in the scalp, face, ear cup, chin, back and legs. These lessions progressively enlarged and later ulcerated. All sibs had severe growth and motor retardation. Histopathological evaluation of these masses revealed homogenous eosinophilic matrix with single cells or cords of spindle shaped cells. Histochemically, the extracellular matrix was periodic acid schiff (PAS) positive and diastase resistant.Alcian blue, toluidin blue and congo red were negative. In reticulin preperation, the cellular areas were rich with reticulin fibers. Immunohistochemically, vimentin was strongly expressed in both spindle shaped and single round cells. In some areas, these cells also showed actin positivity suggesting myofibroblastic nature. Altogether these findings support JHF diagnosis. Interestingly, myeloid cells show an apparent intracytoplasmic vacualization in these patients. Neither a locus or a gene have been reported for JHF as yet. Investigating homozygosity in inbred families is a very efficient tool to identify new genetic localizations. Thus, this family with 10 informative meiosis will be a good source to identify JHF locus using homozygosity mapping.

P0224. Parkes - Weber - Klippel - Trenaunay Syndrome

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The syndrom Parkes - Weber - Klippel - Trenaunay is a heterogenic clinical unit of hemihypetrophia of the limb and the adequate part of body, angiodysplastic changes with varices and other facultative characteristics.
Hemangiomatosis and affections of the skin, soft tissues and adjacent bones and a partial hypertrophy (mainly of the lower limbs) are the substance of the disease.
The Parkes - Weber - Klippel - Trenaunay syndrome is an associated mesodermal and ectodermal dysplasia of congenital and polygenic character.
The authors investigated the group of 23 patients with this diagnosis for a long period of time. There were provided the complex pediatric and genetic examinations, including genealogy and anthropometry. Phenotypic characteristics of this biomechanically important disease were photographically documented.
The genealogic examination found the microsymptoms in the families, such as varices cruris.
The genetic examination has a specific position in the complex of the clinical examinations. It estimates the risk of the affection in offsprings and brothers and sisters, i.e. the relatives of the 1st. degree.
The majority of the cases were isolated with good genetic prognosis. Rarely we found the transmission in two generations with remarkable deviations.
The patients are often treated for other /symptomatic/ diagnosis. Biomechanical and therapeutical aspects of the disease are discussed.

P0225. Secular Trend: Better Growth Of Indian Thalassemia Major Patients Compared To Old Indian Growth Standards

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Beta Thalssemia major is one of the commonest single gene disorder in India with a mean prevalence of 3.5 %. Short stature and delayed puberty, which affect individual's personal image, are common complications associated with thalassemia major (TM) patients. Such problems have psychological implications which deter an individual from participating in peer group activities. In developing countries, thalassemic patients are at a high risk of developing endocrine deficiencies and hence growth failure. We evaluated growth pattern of TM patients to see if they are experiencing secular trend in height and weight as has been observed in the world populations.
Material: Height, weight, serum ferritin and pretransfusion hemoglobin of 90 patients (2-17 years) were evaluated over a period of 3 years. Growth pattern of these patients was compared with New and Old Indian growth Standards and thalassemic patients from New Delhi.
Results: Growth pattern of patients shows that they were significantly shorter than New Indian Growth Standards . There was no significant difference between height and weight of our patients and thalassemic patients from New Delhi. Although our patients above the age of 11 years were short, the prepubertal patients were taller and heavier than 35 years old Indian growth Standards. This suggests that our patients are experiencing secular trend in height and weight.
Conclusion: We attribute gain in height and weight to better treatment regime. Better treatment and management can improve growth of TM patients. However, this is possible provided financial support from national and international agencies is available.

P0226. Molecular characterization of an inv(6)(p12q16) in a girl with CHAR syndrome

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CHAR syndrome, first described in 1978, is an autosomal dominant disorder comprising facial dysmorphy, digital abnormalities and patent ductus arteriosus (PDA). Facial dysmorphy seems to be the major criterion to establish clinical diagnosis: wide-set eyes, ptosis, strabismus, flat nasal bridge, short philtrum, large triangular mouth with duck-bill lips, low-set ears). An incomplete penetrance of the patent ductus arteriosus is observed in several families, intelligence is usually normal. Missense mutations with dominant negative effect in the TFAP-2ß gene localized in 6p12 were recently described. TFAP-2ß encodes a transcription factor expressed in neural crest cells, it was shown that this gene is also involved in ductal, facial and limb development in the mouse. We report the case of a young woman born in 1978 who presents with a typical CHAR syndrome facial dysmorphy without any cardiac anomalies. The karyotype shows a pericentric inv(6)(p12q16), the breakpoint on 6p is located within the region 6p12 harboring the TFAP-2ß gene. Using FISH, we have demonstrated that the TFAP-2ß locus is disrupted by the 6p12 breakpoint, however we did not detect any junction fragment by Southern blot analysis with the TFAP-2ß cDNA. We assume that the breakpoint is close but outside the TFAP-2ß coding region, the use of pulse field gel electrophoresis should clarify this point. This young woman recently underwent amniocentesis for prenatal chromosome analysis for her first pregnancy. The foetus inherited the maternal inv(6)(p12q16) and ultrasonic scan showed the same phenotype as observed in the mother (facial dysmorphy without cardiac anomalies).

P0227. Syndactyly type-I: study of six large Indian pedigrees with variable expression

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Hereditary syndactyly was classified into five different types. Syndactyly type-I (SDTY1) (OMIM 185900) involves complete or partial bilateral syndactyly between third and fifth fingers which is occasionally associated with fusion of the distal phalanges. Feet are rarely affected. It may be an isolated condition. The genes responsible for SDTY1 and SDTY2 have been mapped to chromosome 2q34-q36 (Am J Hum Genet 67:492-97, 2000; Am J Med Genet 104:147-151, 2001 and 2q31 (Hum Molec Genet 4: 1453-1458,1995), respectively.We have studied six large Indian pedigrees with an autosomal dominant SDTY1. Pedigrees consist of 157 individuals, including 65 affected (31-males/34 females). Severity of the phenotype was quite variable among the families and no skipping of generation was observed. In five families, 39 members were bilaterally affected with typical features of syndactyly type-1 affecting the 3rd and 4th fingers and 18 members had only unilateral findings. Few of these also had unilateral partial syndactyly of 2nd and 3rd toes. In the sixth family, complete unilateral or bilateral syndactyly affecting 3rd, 4th and 5th fingers was observed. Few of the affecteds in this family had unilateral elongation of 2nd and 3rd toes with syndacytly. Phalangeal bones were not affected in any of

these families, eventhough the nails are involved in 15 affecteds. Linkage studies with markers closely linked to SDTY1 and SDTY2 will either confirm allelism to these loci or provide evidence for genetic heterogeneity.

P0228. Aortic root dilatation is not demonstrated in EDS patients.

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EDS (Ehlers Danlos Syndrome) is commonly mentioned in the context of heritable connective tissue diseases with cardiac involvement. Among these abnormalities aortic root dilatation is frequently reported. Unlike in Marfan patients, where aortic root dilatation is one of the major criteria, literature data in EDS patients are controversial.
We report on aortic root diameter measurement with echocardiography in a prospective cohort of 40 patients with established EDS. According to the clinical criteria from the Villefranche Nosology (1) 6 were classified with classical EDS, 23 with hypermobile EDS and 11 with vascular EDS.
The mean age of the patients was 24.7 (4-41) yrs and 30% were male. Mean BSA was 1.54m²
Mean aortic diameter at the sinuses of Valsalva was 26.9mm (SD 4.6mm). Mean aortic diameter at the supra-aortic ridge was 23.8mm (SD 3.69mm).
For each patient we compared the aortic root diameter with the population based norms (Roman et al (2)). None of the patients showed aortic dilatation at the sinuses of Valsalva. One 17y old female patient with hypermobile EDS had an aortic diameter at the supra-aortic ridge that was slightly higher than 2SD
In conclusion, we could not demonstrate aortic root dilatation in our series of 40 patients with EDS. This is important with respect to the management and genetic counselling in EDS. Longitudinal follow up studies are necessary to evaluate the evolution of aortic root diameters in this patient group.
1. Beighton et al, Am J Med Genet;77(1):31-7.
2. Roman et al, American journal of cardiology;64(8):507-12.

P0229. Clinical phenotype of a familial translocation t(3 ;5)(p23 ;p14) with partial trisomy 3p2 and partial monosomy 5p.

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Trisomy3p and monosomy 5p are characterized by facial dysmorphism, mental retardation and malformations.
We report two siblings with partial trisomy 3p2 and partial monosomy 5p.
The first baby was a stillborn female with pulmonary hypoplasia, intrauterine growth retardation. She presented with facial dysmorphism, flat face, hypertelorism, large anterior fontanel, almond-shaped eyes, simian palmar crease, abnormal cerebral maturation, hepatomegaly and bilateral renal cysts. Familial history was negative.
The second child was a male born with hypotrophy, auricular septal defect, genital hypoplasia, cryptorchidism and hypospadias, microcephaly, partial corpus callosum agenesis and brachydactyly. Facial dysmorphism was obvious with a flat face, hypertelorism, large forehead, small nose, short filtrum, thick everted inferior lip, microretrognathia, short neck, and dysplastic ears. At the age of 6 years, he had a severe mental retardation, no language but a normal growth.
Both cases in the same family were suggestive of a cryptic chromosomal anomaly as the standard caryotype was normal. Extensive metabolic screening was normal but high resolution caryotype with incorporated BrDU revealed a cryptic translocation confirmed by 3 and 5 chromosomal painting and specific FISH, due to a non-balanced translocation transmitted by the maternal grandfather : 46, XY, der(5)t(3 ;5)(p23 ;p14)mat)
We compared the clinical phenotype to other trisomy 3p and monosomy 5p cases and observed marked trisomy 3p2 clinical features as described in other cases where this region is also involved with other chromosomal partners. This observation will help establish the correlation of this critical chromosomal region and the clinical features.

P0230. A new case of terminal 2q37 deletion diagnosed in adulthood.

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Among terminal 2q37 deletion, very few cases (Reddy et al 1999) have already been described in adulthood. We report on one young female, 19 years old, with mild mental delay, obesity, joint hyperlaxity. The diagnosis was suspected because of dysmorphic features leading to Albright Hereditary Osteodystrophy (AHO) - like phenotype. The phosphocalcic level and routine resolution karyotype were normal in blood, but this de novo 2q37 deletion was confirmed by a specific subtelomeric 2q probe (FISH).
This patient might help us to give more specific informations, as medical follow-up, to parents of an affected young child, even if the range of clinical findings is wide. In fact, we compare our patient with cases already reported in litterature.

P0231. Glomuvenous Malformation (“glomangioma”) Is A Distinct Clinicopathologic And Genetic Entity

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Glomuvenous malformation (“glomangioma”) (GVM) is a cutaneous vascular lesion histologically characterized by ectatic vein-like channels with mural “glomus cells”. Discovery of the molecular basis for GVM permitted delineation of the clinical phenotype and comparisons between GVM and other venous anomalies.
Clinical data based on physical examination, and review of medical records and clinical slides was compiled for 1716 patients with inherited or sporadic venous anomalies. Diagnosis was histologically and/or genetically confirmed for all inherited venous lesions and most sporadic lesions. The data were statistically analyzed using the Fisher’s exact test (two-tailed) with SYSTAT software (version 10, http:// www.spssscience.com/systat).
We identified 30 patients with sporadic GVM, 105 with inherited GVM, 1548 with sporadic venous malformation (VM), and 33 with inherited mucocutaneous venous malformation (CMVM). GVMs, accounting for 5% of venous anomalies, were frequently inherited (78%), whereas venous malformations were rarely familial (2%). GVMs always involved skin and subcutis, in contrast to CMVMs and VMs ($p<0,001$). GVMs had a distinct raised cobblestone appearance. Trauma was reported as an inciting factor for appearance of new lesion by patients with inherited GVM ($p=0,007$), in contrast to patients with CMVM. None of the patients with extensive GVM presented with coagulopathy, in contrast to patients with large VM.
This is the largest series of patients with cutaneous venous anomalies. It establishes clinical criteria for differential diagnosis between GVM and other subtypes of venous anomalies. This differential diagnosis is essential as the treatment and outcome are different. (vikkula@bchm.ucl.ac.be)

P0232. Inherited Capillary Malformation Is Associated With A High Flow Vascular Malformation

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of Cellular Pathology, Université catholique de Louvain, Brussels, Belgium.
Capillary malformation (CM) is a common cutaneous vascular anomaly occurring in 0,3% of neonates. Except for rare reports of familial nuchal CM ("stork bite"), CM is considered sporadic. Clinical diagnosis is usually straightforward, however, CM can masquerade a high-flow vascular lesion, i.e., stage one AVM or AVF. We identified several families with inherited CM. We clinically examined each family member, noted all vascular anomalies and collected informed consents and blood samples.
Inter- and intrafamilial variation was observed, from nuchal CM persisting throughout life and extending below the hairline, to solitary or multiple CM disseminated all over the body. Seven of our families with inherited CM had a member affected with a high-flow vascular malformation: three with either AVM (n=2) or AVF (n=1). Four families had two members with AVM (n=8) including Parkes Weber syndrome (n=3), AVM with multiple CM (n= 3), and intraosseus AVM with cutaneous blush (n=1) or with extensive CM (n=1).
These data confirmed autosomal inheritance of CM. The high incidence of high-flow vascular malformations within families with inherited CM suggests genetic predisposition for AVM and/or AVF in these families. (vikkula@bchm.ucl.ac.be)

P0233. Chromosomal aberration in a girl with craniosynostosis syndrome.

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We describe a case of a girl with craniosynostosis syndrome and finding of chromosomal aberration.
Fenotype: Severe failure to thrive, psychomotoric delay, hypotonia, skull malformation (craniosynostosis of sagittal and coronal sutures). Facial features consists of severe coloboma of the right palpebrae, high prominent forehead and frontal bossing, flattened face with beaked nose, long philtrum, narrow lips, dysplastic ears, preauricular pit. Other features were clinodactyly of the fifth fingers, umbilical hernia, ventricular septal defect and optic nerve atrophy.
In our index case a 46,XX, del (10) (q25-qter) was proven by cytogenetic analysis.
In this region gene FGFR2 is located , mutation of this gene results in various disorder: Crouzon sy, Pfeiffer sy, Jackson -Weiss, Apert sy, Seather Chotzen sy.
We assume that the phenotype in our patient is in causal relationship with the chromosomal 10 q deletion. However, cases previously reported in the literature had less dysmorphic features, than our propositus. Possible explanation of this observation will be discussed. Molecular cytogenetic methods and DNA analysis of the critical region is further proceeding.

P0234. Cardiofaciocutaneous syndrome: molecular aspects

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Cardiofaciocutaneous (CFC) syndrome is a rare disorder described by Reynolds et al.[1986] in eight unrelated patients with very similar facial appearance, mental retardation, failure to thrive, congenital heart defect, short stature and ectodermal abnormalities. All patients diagnosed with CFC syndrome are sporadic, born to non consanguineous parents and had normal chromosomes on conventional cytogenetics.
Rauen et al. [2000] reported on a patient with CFC phenotype and an interstitial deletion of the 12q21.2q22 chromosome region. The authors concluded that a gene responsible for the CFC syndrome is likely to reside in this chromosome region. We looked for microdeletions within this region in 17 CFC patients (12 males and 5 females), aged 2 to 25 years, by FISH, using 12 BAC probes covering the 12q21.2q22 region at average intervals of about 1 Mb. The patients presented with mental retardation (17/17), typical facial appearance, with high forehead, bitemporal constriction, bulbous nose, low set and posteriorly angulated ears, palpebral ptosis and downslanting eyes (17/17), ectodermal abnormalities, consisting of thin, sparse and curly hair, absent or sparse eyelashes and eyebrows, keratosis pilaris, or hyperkeratotic skin patches (17/17), speech delay (17/17), short stature (16/17), heart defect (14/17) and relative macrocephaly (12/17). No microdeletion was detected in any of the patients. Based on these results, we conclude that CFC syndrome is unlikely to be caused by a microdeletion in the 12q21.2q22 region. We suggest that Rauen et al.'s case could represent a different chromosomal syndrome, with some phenotypic resemblance to the CFC syndrome.

P0235. Computer-Assisted Recognition of Syndromic Faces

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Syndromes are often defined by a specific facial appearance. Experienced geneticists usually make a diagnosis through immediate pattern recognition. We have investigated how well a computer can do this. In view of the fact that patients with a syndrome look more similar than unrelated individuals do, we have chosen a pattern recognition program that was developed to identify a person by matching his face to faces stored in a database. It is not based on anthropometric measurements, but uses digital photographs of 256x256 pixels which are subjected to a Gabor Wavelet Transformation to create a vector with 40 complex coefficients (jet) for every pixel. For the purpose of this study, each face was automatically labeled with 48 nodes. The jets attached to each node of a face were then compared to the jets of all nodes at the same fiducial points of every face in the data base (bunch graph). Classification was based on a majority decision of all analysed nodes of a face (jet voting). Analysis of 32 innerfacial nodes from 55 frontal view photographs of patients with mucopolysaccharidosis type III (n=6), Cornelia de Lange (n=12), fragile X (n=12), Prader-Willi (n=12), and Williams-Beuren syndrome (n=13) revealed correct syndrome recognition in 42/55 (76%) of the patients. In another four patients (7%), a correct and an incorrect diagnosis scored equally well. Our results indicate that it may be feasible to develop a program which may aid the clinical diagnosis of genetic syndromes and the study of genetic variation of facial patterns.

P0236. MPZ and PMP22 genes mutations in Croatian patients

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Charcot-Marie-Tooth (CMT) disease is the most common genetic disorder of the peripheral nervous system. The major CMT1 form is associated with 1.5 Mb tandem duplication in band 17p11.2-p12 containing the gene for the peripheral myelin protein 22 (PMP22). Point mutations have been found in PMP22, myelin protein zero (MPZ) (CMT1B phenotype), connexin 32 (Cx32) (CMTX phenotype) and early-growth response 2 (EGR2) genes which have important role in the CMT pathogenesis.
Aim of this study was to investigate mutations in MPZ and PMP22 genes in Croatian patients with different neuromuscular and neurodegenerative diseases.
We employed single-strand conformational polymorphism analysis (SSCP) for mutational screening. PCR products with altered mobility patterns were sequenced to analyze the type of mutation.
A novel Ser8Ser mutation was found in exon 1 of the MPZ gene in two heterozygous subjects, in a father with mild CMT2 phenotype and his daughter with normal clinical data. Thr118Met polymorphism was found in exon 5 of the PMP22 gene. For the first time we found heterozygosity for 118Met allele with nemalin myopathy. The second heterozygous patient for 118Met allele had CMT1 disease.
We conclude that the occurrence of the 118Met allele does not usually cause CMT1 and is not a clinically relevant disease marker.

P0237. Kabuki make-up syndrome in three Turkish Patients

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Three Turkish patients, a female and two males; 15, 8, 2 years of age with Kabuki Make-up syndrome were reported. All three individuals had the cardinal features of the syndrome such as motor and mental retardation, peculiar facies including long palpebral fissures, large malformed ears, depressed nasal tips. Two cases showed postnatal growth retardation; one case had a history of recurrent otitis media, all they had normal chromosomal composition. In one of them pedigree analysis indicated that the condition showed autosomal dominant inheritance with variable expression. .
Additionally cranial MRI of one of them revealed hypoplasic corpus collosum and periventricular leukomalasia which has not been described before in this syndrome.

P0238. Antigliadin antibodies and antiendomysial antibodies in Down syndrome children

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Celiac disease, also known as gluten-sensitive enteropathy is a chronic inflammation disease of the small intestinal mucosa. Detection of antigliadin and antiendomysial antibodies in serum is important in diagnosis and screening of celiac disease. Ig A antiendomysial antibodies have greater sensitivity compared to antigliadin antibodies. It has been reported that the prevalence of celiac disease is higher in Down syndrome children as the other autoimmune conditions. In this study, 32 Down syndrome children (22 male / 10 female) have been evaluated for antigliadin antibodies and antiendomysial antibodies. Total blood count and immunglobuline values were in normal levels. There was no known disease or no abnormality in biochemical levels at entry into the study. [The youngest was 2 year-old and the oldest one was 17 year-old (average: 6.55±3.88)]. None of the 32 children had known celiac disease at entry. Six out of 32 patients (18.75%) were found to be serological positive, 5 (15.63%) were found to have antigliadin antibodies' levels above normal; and 5 (15.63%) to be antiendomysial antibodies positive. In four patients (12.5%) both AGA Ig A levels were above normal and AEA Ig A were positive. One patient (%3.3) was only AGA Ig A positive, and one (%3.3) was only AEA Ig A positive. Duodenal biopsies of two cases out of 6 serological positive cases showed villous atrophy (males with both AEA and AGA positive), and two cases revealed normal mucosa (male with both AEA and AGA positive, male with only AEA positive).

P0239. Clinicopathological features of the foetus with 21q-.

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A male foetus with multiple malformation was found by ultrasound examination at 25-26 weeks` of gestation. Cytogenetic investigations revealed a 46,XY,del(21)(q22) karyotype in of the umbilical cord blood lymphocytes. Molecular analyses showed that the deletion occurred on the maternal chromosome between markers IFNAR and D21S1255, including "critical region" of Down`s syndrome (21q22.3). In view of the poor fetal prognosis, the woman elected pregnancy termination. The autopsy revealed: intra-uterine growth retardation, hydrocephaly, facial dysmorphy characterized by microcephaly, hypertelorism, low-set deformed ears, micrognaty, microphthalmy, dextrocardia, polycystic lungs, skeletal abnormalities including deformity of the chest, 13 ribs on right, flexion contractures of the right foot, deformities of the fingers, hypoplastic fallus bound by chordee, hipoplastic scrotum.
The histological pictures of the different fetal tissues showed pathological immaturity of the liver, kidney, suprarenal glands, lien, hypoplasia of eye chambers. The lung was contained of the multiple cyst cavities of different forms. The placenta showed the indication of pathological differentiation of villous maturation with frequent persistence of embryonic forms of villi, which are non specific lesions and can be observed in a variety of other conditions.

P0240. Donohue syndrome (MIM 246200) in a boy with 13q12.3-q21.2 deletion.

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We report on a boy showing features suggesting Donohue syndrome (MIM 246200) and a deletion on 13q12.3-q21.2 region, seen by standard karyotype. After an uneventful pregnancy from unrelated parents with a negative family history, he was born small for gestational age. Developmental delay has been referred from the very beginning. Failure to thrive not caused by celiac disease and poor growth continued up to 3 years when a diagnosis of growth hormone impairment was made. Before this diagnosis he had two sudden episodes of fasting hypoglicemia, which continued during treatment with recombinant growth hormone, up to the last one reported 4 months ago. We have observed him for the first time at the age of 7.9 years. He has moderate mental retardation, short stature, long elfin-like face with large everted ears, thick lips and gum hypertrophy, joint hyperlaxity and wrinkled loose skin. Donohue syndrome has been already associated to mutations in the insulin receptor gene, on 19p13.2. The 13q12.3 region is the breakpoint of our deletion on the centromeric side, but also the region where the insulin-regulating transcription factor CDX3 (MIM 600297) maps. CDX3 works as homeodomain protein binding an A/T-rich sequence in the promoter of the insulin gene (MIM 176730). We hypothesize that the deletion of CDX3 may be involved in the clinical features of our patient and provide a new candidate for the genotype of Donohue syndrome.

P0241. 3p- Syndrome in a girl with an apparantly balanced translocation (3;5)

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The 3p- syndrome results from deletion of a terminal segment of the short arm of one chromosome 3, and is characterized by mental retardation, growth failure, hypotonia, and multiple congenital anomalies, such as microcephaly, blepharoptosis, hypertelorism, ear anomalies, heart defects and deafness. The constellation of symptoms depends on the extent of the deletion.
Most deletion breakpoints are at 3p25-26, and among the genes mapped in region 3p25-3pter are ATP2B2 (involved in cardiac development), the VHL gene (involved in Von Hippel Lindau disease) and the CALL gene (possible role in brain development).
In most cases of 3p-syndrome the deletion arose de novo.
We describe a female patient who was presented to us soon after birth showing the following features: intrauterine growth retardation, single umbilical artery, hypotonia, microcephaly, blepharoptosis, hypertelorism, micrognathia, low set ears, ear pits, a long philtrum and a thin upper lip. At follow up at the age of 6 months an 1 year she showed severe reflux, developmental delay, and a thickened artrial valve at ultrasound of the heart.
Chromosomal analysis showed an apparantly balanced translocation (3;5)(p25;q31.1) inherited from the healthy father. Further subtelomeric studies of the patient showed a terminal deletion of the short arm of chromosome 3. Possible mechanisms are discussed and the importance of subtelomere studies is stressed in apparantly balanced translocations with very small segments involved.

P0242. Craniosynostosis, branchial cyst and inguinal calcifications, an unusual presentation of AHO (Albright hereditary osteodystrophy)

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Albright hereditary osteodystrophy (AHO) is characterized by phenotypic signs that typically include brachydactyly and subcutaneous calcifications occurring with or without hormone resistance toward PTH or other hormones such as thyroid hormone or gonadotropins. We were presented a male dysmature baby born after 36 weeks of pregnancy. On exam, he showed a trigonocephalic, asymmetric skull, abnormally formed orbits, a branchial cyst and subcutaneous calcifications around the ankles and in the inguinal

region. Laboratory investigations showed a transient hypoglycemia and a hypothyroidism. The patient was treated with L-thyroxine and had an operation of a metopic suture synostosis. The diagnosis of AHO was confirmed by identifying a mutation (701G→A)in the GNAS1 gene. While the majority of symptoms of our patient are typical for AHO, to our knowledge, a branchial cyst has not yet been reported in AHO. Coincidence or genetic relationship will be discussed and a follow-up of the patient will be presented.

P0243. Ectrodactyly in the Genetics Clinics

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Ectrodactyly is encountered in mendelian traits as well as in rare chromosomal anomalies and as sporadic. The anomaly occurs during organogenesis and recently its pathogenesis is better understood (Wolpert, 1999). Retrospective analysis of the 48 probands with confirmed or suspected ectrodactyly evaluated at the USF genetics/dysmorphology and prenatal clinics between 1/2/82 and 12/31/00 is presented. The probands were part of the 38,706 probands/families evaluated during the period. They were retrieved using patient database. Ectrodactyly/split hand was one of the 3 primary diagnoses. Four of the 48 were no shows in the clinic and were excluded. There were 27 males and 17 females. Twenty-two of 44 were Caucasian, 9 Hispanic, and 9 African-American matching the racial ratio of the population of West Central Florida. Typical lobster claw anomaly type I was present in 19 and atypical in 25. Two had chromosome anomaly, t(13q14q) with maternal class A 1 diabetes mellitus in one and another with complex chromosome rearrangement with 6 breaks on chromosomes 2,3,5,11, and 13 resulting in interstitial deletion 13q. Two probands had mothers with diabetes mellitus but no other anomalies to diagnose diabetic embryopathy. Eleven of the 44 had positive family history indicative of autosomal dominant trait with reduced penetrance. Fourteen of the 44 had associated anomalies implying syndromes. Among them were 3 probands with ectrodactyly-ectodermal dysplasia-cleft palate. This study showed that ectrodactyly has 1. varied phenotypes difficult to classify and 2. is genetically heterogeneous. As to a candidate gene only the probands with chromosomal abnormalities implied chromosome 13.

P0244. Cockayne syndrome type II

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Cockayne syndrome/CS/ is an autosomal recessive dysmorphic syndrome,caused by defects of transcription-coupled DNA repair due to truncating mutations, point mutations, or deletions of CSA(chr.5) and CSB(10q11-q21) genes.CS manifests a wide clinical spectrum including CS type I, the “classical” form; CS type II(connatal form), a more severe form with symptoms present at birth,CS type III, a milder form; and xeroderma pigmentosa-Cockayne syndrome (XP-CS).Patients with “connatal” CS have evidence of growth failure at birth, with little or no postnatal neurological development. Congenital cataracts or other structural anomalies of the eye as well, as arthrogryposis or early postnatal contractures of the spine (kyphosis, scoliosis) and joints may be present.This group overlaps clinically with two genetic conditions bearing different names, the cerebro-oculo-facial syndrome (COFS) and Pena-Shokeir type II syndrome. The authors present clinical observations of a 3 year old girl/DOB-24.04.98/,second child of healthy,unrelated parents/father-30,mother-32/.The girl was born 18 days before term/W-1900 gr.,L-44 cm./ and manifested developmental delay and repeated respiratory infections during the first year of life.Photosensibility has been noted at 11 months.At 2 2/12 yrs. she is underweighed/wSDS - -2.71/ ,microcephalic/hcSDS -3.5/,with short neck,dysmorphic face,mild thoracic kyphosis,unsteady gait and moderate mental retardation/IQ - 44/.Abnormal retinal pigment distribution has been detected.Routine laboratory investigations,chromosome analysis and CT scan showed normal results.Cockayne,Bloom,Rothmund-Thompson and COFS syndromes have been discussed.DNA repair studies on skin fibroblasts disclosed strongly inhibited DNA synthesis after UV-irradiation,thus confirming the diagnosis of CS.Mutation analysis is in progress.

Imrovment of motor development and cognitive functions has been achieved during 1 year follow-up and symptomatic treatment.

P0245. Clinical and molecular genetic investigation of the classic type of Ehlers-Danlos syndrome in Russia

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Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders characterized by skin hyperextensibility, impaired wound healing, joint laxity, tissue fragility, skeletal deformities and other changes. “Classic” EDS type (Villefranche Nosology, Beighton et al., 1997) which includes EDS types I (MIM 130000) and II (MIM 130010) is the commonest variant of the EDS with genetic heterogeneity. Mutations in type V collagen, COL5A1 and COL5A2 genes, have been shown to underlie this type of EDS in some cases. Thirty four patients from 21 families with diagnosis of EDS I or II types and their 21 relatives were clinically investigated. To evaluate the role of COL5A2 gene mutations in this group of patients skin biopsy samples were taken from 6 patients with EDS I and 5 patients with EDS II. We isolated mRNA from cultured fibrobasts and made RT-PCR to get cDNA . Eleven pairs of primers for about 4,5 kb mRNA COL5A2 were designed and carried out the PCR-SSCP analysis. We have found the absence of first fragment including the first ATG codon in EDS II patient. The clinical signs included thin skin with moderate hyperextensibity, easy bruising, joint laxity, scoliosis, pectus excavatum, flat feet, myopia, retinal bleeding and varicose rectal veins. Other manifestations were striae and recurrent hyperbilirubinemia, which are not typical for EDS. The study is in a progress now.

P0246. The natural history of human dermatosparaxis (Ehlers-Danlos Syndrome VII C)

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Ehlers-Danlos Syndrome (EDS) type VIIC (human dermatosparaxis) is a recessively inherited connective-tissue disorder, characterized by extreme skin fragility, premature rupture of the membranes, umbilical hernia, blue sclerae, characteristic facies and joint laxity. Like the animal model it is caused by defects of procollagen-I-N-proteinase-activity, resulting in an inability to cleave the N-terminal propeptides of procollagen-I-N-chains. Electron microscopy typically consists of a characteristic “hieroglyphic” pattern of collagen fibrils. To date only 7 human cases have been recorded, most of them being aged under 2 years. We report the clinical follow-up history of one of the first reported patients (20 months;Nusgens et al, 1992). Now, at the age of twelve, marked skin fragility and easy bruisability continue to predominate the clinical picture. During the past years, she encountered several problems due to extreme tissue fragility, such as spontaneous bladder rupture, persisting bladder diverticulae with recurrent urinary infections, and rupture of the diaphragm with para-esophageal hiatus hernia after vomiting. On physical examination, she presents with an old appearance, with sagging skin and excessive wrinkles in her face and on hands and feet. Her stature is below P3. Joint hyperextensibility is very mild. She presents several unusual oral and dental anomalies. Being of normal intelligence, she was confined to home education untill the age of ten because of her extreme vulnerability. Now she attends a school for physically handicapped children.
EDS VIIC appears to be a severe debilitating disease due to lifelong skin and tissue fragility.
Nusgens et al, Nat Genet 1:214-17, 1992

P0247. Locus heterogeneity in lymphedema-cholestasis syndrome (Aagenaes syndrome)

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Lymphoedema – cholestasis syndrome (LCS, Aagenaes syndrome, MIM 214900) is the only form of hereditary lymphoedema associated with cholestasis. Most patients are of Norwegian origin. A locus for LCS, LCS1, has recently been mapped to chromosome 15q in the original extended kindred reported by Aagenaes and coworkers. We have studied a family of Serbian gypsies in which a child born to consanguine parents had LCS, and performed linkage and haplotype analysis at marker loci (D15S979, D15S202, D15S996, D15S127, FES, IP15M9, D15S158, D15S963, D15S652) spanning the LCS1 region on 15q. This excluded the disease locus from this region, implying locus heterogeneity for LCS.

P0248. Bilateral Congenital Ptosis and Variable Toe Anomalies: A New Autosomal Recessive Syndrome?

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Congenital ptosis with digital anomalies among other manifestations has been reported in a variety of syndromes. Here, we describe two Saudi Arabian brothers with congenital ptosis and variable toe anomalies including hypoplasia, clinodactyly, bifid great toe, partial syndactyly between the second and third toe, overriding toes, and absent toe nails. Both children have displayed normal growth and development with no evidence of other system involvement. Their karyotypes were normal. The parents are first cousins and phenotypically normal. An older brother has an isolated ptosis with no digital anomalies.
We think this constellation of anomalies in this family represents a new autosomal recessive entity with variable statement.

P0249. Clinical and genetic heterogeneity and ethnic differences in Bulgarian FSHD patients

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FSHD is an AD inherited desease, associated with a deletion within the telomeric portion of chromosome 4. The gene responsible for development of the disease is still unknown. FSHD presents with a high degree of clinical variability, with respect to age of onset, severity and pattern of muscle involvement. Twenty patients from 7 families and eight sporadic cases were studied. The families were of different ethnic origin- bulgarian, turkish and gipsy. The disease started before the twentieth year of age in 90 % of cases. We determined significant inter- and intrafamilial clinical variations in the course of the FSHD. Some of our families showed phenomenon of anticipation. In most of the cases additional features, like cardiac disturbances, deafness and retinal vasculopathy were found. Mental retardation and schisophrenic psychosis were specified in the members of the two most severely affected turkish families. Some of the patients presented with a phenotype of limb-girdle muscular dystrophy or another unusual phenotype.
Some of the cases were genetically determined as being connected with the FSHD1 locus in 4q35. Sizes of the fragments were inversely connected with the clinical severity of the disease. Several patients were not connected with the locus, which instituted genetic heterogeneity of FSHD for Bulgarian patients.

P0250. Three novel AR neuropathies in Bulgarian Gypsies

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For variety of social and cultural practices various Gypsy groups in our country represent genetic isolates with a very high rate of some autosomal recessive neuromuscular disorders**.** During the last 7 years we identified several single gene disorders limited to specific traditionally endogamous Gypsy groups in Bulgaria:
1. Hereditary motor and sensory neuropathy type Lom (HMSNL) is a severe demyelinating peripheral neuropathy associated with sensorineural deafness, mapped to chromosome 8q24 (Kalaydjieva et al., 1996, 1998) and caused by a truncating mutation in the N-myc downstream-regulated gene-1 (Kalaydjieva et al, 2000). The disorder affects different endogamous Gypsy metagroups belong to the Wallachian Gypsy group: Kalderas, Kopanari and Lom Gypsies.
2. Congenital cataracts facial dysmorphism neuropathy (CCFDN) syndrome is a novel multisystem genetic disorder which combines both developmental abnormalities and a progressive neurological deficit (Tournev et al., 1999). The most important features of the syndrome include congenital cataracts and microcorneae, dysmorphic facial features and small stature, mental retardation, a predominantly motor peripheral neuropathy and hypogonadotropic hypogonadism. The CCFDN syndrome was mapped on 18q23 (D. Angelicheva et al., 1999).
3. Hereditary motor and sensory neuropathy type Russe (HMSNR) is a severe, progressive, sensorimotor neuropathy with prominent sensory loss in association with an intermediate reducton in nerve conduction velocity (P.K. Thomas et al., 2001). Linkage analysis localized the HMSN-R gene to chromosome 10q23, in close proximity to the EGR2 gene (T. Rogers, 2000).

P0251. A Jagged 1 gene mutation for abdominal coarctation of the Aorta in Alagille syndrome

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Structural cardiac defects such as peripheral pulmonary stenosis are well described in Alagille syndrome (AGS), which is transmitted in an autosomal dominant inheritance. Haploinsufficiency of the Jagged 1 gene (JAG1) has been shown to cause Alagille syndrome. Abdominal coarctation is an uncommon vascular congenital anomaly and has been described only twice in AGS. We report on two more patients with coarctation of the abdominal aorta in AGS.
Recently, Loomes et al (Hum-Mol-Genet. 1999; 8(13): 2443-9) have shown that Jagged1 gene is expressed in the developing heart and in multiple associated vascular structure including the descending aorta. Jones et al (J Med Genet 2000;37:658-662) studied the tissue expression of JAG1 in human embryo and noticed that JAG1 was also expressed in the developing aorta. We performed molecular analysis of the Jagged1 gene in one of our patients with AGS and abdominal coarctation and found a mutation deletion (1485 Del CT). This corroborates with Loomes and Jones studies and confirms that coarctation of aorta might be a part of the Alagille syndrome. This additional report brings the number of children with Alagille syndrome and coarctation of abdominal aorta to four and emphasizes the need to follow children with Alagille for the development of coarctation of the abdominal aorta.

P0252. Two cases of coincidence of monogenic autosomal linked neurodegenerative disorders and the chromosomal abnormality of gonosomes.

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We present here two cases of autosomal monogenic disorder

in combination with numeric failure of gonosomes. Though the coincidence of abnormality of gonosomes and X-linked neurodegenerative disorder is not unexpected finding, the combination with autosomal gene disorder is quite rare.
The first case is the four-month old girl with the symptomatology of spinal muscular atrophy. Chromosomal abnormality 47,XXX was found, and SMN1 gene dysfunction was diagnosed by DNA analysis. The proband died at the age of 8 month.
The second case is a patient followed up from the age of 2 years because of the symptomatology of HSMN and progressive growth retardation. Her maternal grandfather died at the age of 46 having neurodegenerative disorder (HMSN -FRDA??). DNA analysis encrypted the specific duplication of the 17p11.2-p12- the CMT1A. The chromosomal investigation revealed de novo 45,X/46XY (1:2) karyotype.
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P0253. Interdisciplinary strategies design: evaluation of sensorineural hearing loss (SNHL) pedigrees from patients classified as indeterminate. The importance of professional formation

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Prevalence of genetic SNHL is low in Argentina (12%). We attempted to revise the diagnosis of SNHL, according the worldwide accepted classification: genetic, environmental, indeterminate to estimate the actual prevalence of the genetic component, exerting prevention, and guiding of groups at risk, institutions and professionals closed involved. A qualified population of SNHL patients (n=260), aged 0-15yr attending public/private services, and educational institutions of Rosario, is divided as follows: genetic (10%), environmental (51%), indeterminate (39%) groups. Further familial information to draw the corresponding pedigrees was obtained (n=101probands), detecting syndromic and non-syndromic SNHL cases. This investigation provided us with two perspectives: 1) adequate training of the staff to prepare the pedigrees; 2) counselling of subjects at risk. It is to be noted the importance of improving the professional practice to construct a “new professional”. Regarding counselling of subjects at risk it is admitted that: A) transfer of results (diagnosis, prognosis) must warrant privacy, based on psychological, sociocultural, ethical and philosophical principles. B) there is a well known impact produced by “knowing oneself and the own expectations”, thus it is convenient to provide psychological support and/or preserve the subject emotionally, as well as to suggest a consultation with specialists. C) The task must be centred on the patient. We do not adhere to directiveness, conversely, emphasising the patient and/or family -and other subjects at risk- decision. Hence, it is obligatory to organize workshops for professionals focused mainly on prevention and promote the re-enactment of the individual and collective construction of the professional profile.

P0254. Menkes syndrome - clinical study of a family

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We present a case of Menkes syndrome in order to discuss the importance of different clinical features and the management of the patient and his family. Our proband is a 5 Mo old male infant, second child of an young, apparently normal, unrelated couple. Physical examination revealed mild growth deficiency, microcephaly, sparse, hypopigmented, kinky hair, pale skin, expressionless face with full cheeks and hypotonic muscles. Radiologic investigation showed wormian bones in the lambdoid sutures and widened metaphyses of the long bones. Hair microscopic examination showed pili torti. Serum levels of copper and ceruloplasmin were low. Based on the clinical features and specific investigations we have establishes the diagnosis of Menkes syndrome. Detailed differential diagnosis with other XLMR entities and other syndromes with pili torti will be provided. The management of the family will be discussed. In conclusion, we present this case of Menkes syndrome to illustrate a rare entity and to discuss the importance of the features for the diagnosis and the management of the family.

P0255. Chondrodysplasia punctata 1, X-linked recessive (CDPX1) and Ichthyosis associated with 46,Y,der(X)t(X;Y)(p22.31;q11.21) karyotype in the son of a mother carrier of the derivative X chromosome

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We report a new case of CDPX1 and ichthyosis in a newborn with the characteristic phenotype, both clinical and X-ray, of this X-linked recessive disorder. Cytogenetic analysis showed his karyotype to be 46,y,der(X)(p22.31;q11.21). The psychometric testing is not well applicable because of the age of the child, but the psychomotor development was apparently delayed at age 8 months. The STS activity is obviously deficient. The mother is a carrier of the derivative X chromosome and has carrier levels of STS activity: she is somewhat short of stature and has mildly short arms.
FISH studies were performed on metaphase chromosomes using Xyqtel, Xyptel, STS and KALI Xp22.3 region probe with DXZ1 chromosome X control probe (ONCOR). A battery of DNA polymorphic markers was used to localize the Xp breakpoint (DXYS233, DXS996, DXS6837, DXS1139, DXS6834, DXS1130, DXS237, DXS278, DXS987). In the mother we also performed the study of DNA markers from Y chromosome, to well determine the location of the Yq breakpoint in the derivative X chromosome.
The mapping studies indicate the precise location of the well characterised genes, SHOX, CDPX1 (arylsulphatase E), steroid sulphatase (STS), and Kallmann (KALI) genes. Further analyses are in progress to better establish a phenotype-karyotype-genotype correlation.

P0256. Costello syndrome: report of three patients with typical manifestations.

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Costello Syndrome is an extremely rare disorder characterized by growth delay after birth (postnatal), leading to short stature; excessive, redundant loose skin on the neck, palms of the hands, fingers, and soles of the feet; development of benign (non-cancerous) growths (papillomata) around the mouth (perioral) and nostrils (nares); mental retardation; and/or characteristic facial appearance. Other physical features may include the development of dry hardened skin on the palms of the hands and the soles of the feet (palmoplantar hyperkeratosis), abnormally deep creases on the palms and soles, and/or abnormally flexible joints of the fingers (hyperextensible). There is an increased incidence of congenital abnormalities of the heart and thickening of the heart muscle called a cardiomyopathy. Characteristic craniofacial features may include an abnormally large head (macrocephaly); low-set ears with large, thick lobes; unusually thick lips; and/or abnormally wide nostrils (nares). Most cases are sporadic and a significant increase of mean paternal age has been reported, suggesting de novo dominant mutations. The exact cause of Costello Syndrome is unknown. We report three new patients with typical clinical findings and emphasize the importance of clinical experience for diagnosis of this disorder.

P0257. Duplication of intrachromosomal insertion segments 4q32-q35 confirmed by comparative genome hybridization and FISH

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A 35-year-old man with oligozoospermia was referred for chromosomal analysis. In routine cytogenetic analysis, the patient was seen to have an additional material of unknown origin on the terminal region of the short arm of chromosome 4. To figure out the origin of the unknown material, we carried out high-resolution banding, fluorescent in situ hybridization (FISH) and comparative

genomic hybridization (CGH). CGH showed a gain of signal on the region of 4q32-35. By using whole chromosome painting and subtelomeric region probes for chromosome 4, we confirmed the aberrant chromosome as intrachromosomal insertion duplication of 4q32-35. We didn't carry out parent's chromosome analysis, but we predicted one of parents might have a ins(4)(p16q32q35) because the abnormal chromosome was a type of possible recombinants from crossing over in carriers with the between-arm intrachromosomal insertion of chromosome 4. Duplication often leads to some phenotypic abnormalities, however, our patient showed almost normal phenotype except for sperm counts.

P0258. A family with Nievergelt type mesomelic dysplasia - case report

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Mesomelic dysplasia - a heterogeneous group of bone dysplasias with disproportionate shortening of middle segment of limbs. Clinic is variable. AD, AR, X-linked heredity forms have been described. We report family where two offsprings 27 year old son and 24 year old daughter with short height (-2SD). Both patients showed radiologically hypoplastic and shortened tibia and fibula, shortened radius and curved ulna.Madelung deformation, radio-ulnar and tarsal synostosis were diagnosed. Mental development is normal. Both parents were phenotypically healthy with normal height. AR mesomelic dysplasia was suspected and low risk for sick offsprings future children was prognosed.During sons` wife pregnancy fetal tibia shortening was diagnosed sonographically on 18 th week of pregnancy. Parents´s detailed radiological investigation revealed in mother deviation of ulna. Therefore AD mesomelic dysplasia (Nievergelt) type was diagnosed. Newborn baby clinical and radiological investigation confirmed diagnose.Family members careful investigation usually is helpful for correct diagnosis and prognosis during genetic counselling.

P0259. A new polyepiphyseal dysplasia with extreme delay in ossification and normal stature in sibs

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We report on a pair of dizygotic twins born to consanguineous parents, showing both an extreme delay in skeletal maturation (roughly corresponding to a newborn at age 4 10/12), mild metaphyseal irregularities, and a phenotype associating normal to large stature (+1 to + 2 SD) with normal growth pattern, long fingers, generalized small joint hyperlaxity with flat feet, genua valga, and low normal intellectual development. This combination appears to represent a new AR - or possibly XLR - skeletal dysplasia belonging to the polyepipyseal dysplasia spectrum of disorders.

P0260. Identification of differentially expressed genes during development of cardiac hypertrophy in rat

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Cardiac hypertrophy is an adaptive response to chronic increased workload and is associated with changes of gene expression. To identify candidate genes contributing to the initiation or progression of cardiac hypertrophy we have screened differential gene expression in the heart of spontaneously hypertensive rats (SHR) at different stages of their development. Up to 4 weeks after birth the animals are normotensive. Males first develop hypertension around 12 weeks pp and hypertrophy of the left heart ventricle around 26 weeks pp. We established a subtractive hybridization system based on cDNA selection and suppression PCR using mRNA from these stages. Subtractive hybridization using age groups 4- versus 12- as well as 12- versus 26-weeks identified 145 different cDNA clones. Screening of these clones revealed 56 cDNAs as candidates for differentially expressed genes during development to cardiac hypertrophy. Northern blot analysis of 42 cDNAs identified 16 genes to be upregulated in cardiac tissue of SHR in comparison to the wild type Wistar-Kyoto rat. In silico analysis of cDNA sequences identified several known genes which are being discussed already in the context with cardiomyopathy (g-sarcoglycan) or cardiac hypertrophy (acyl-coA dehydrogenase). In addition to these genes our analysis revealed so far 8 novel and unknown genes which are differentially expressed in the heart of SHR. Mapping of these novel genes with respect to rat chromosome segments harboring known QTLs for cardiac hypertrophy and analysis of their expression pattern might be relevant to understand processes leading to cardiac hypertrophy in rat and man.

P0261. High prevalence of a screening detected, HFE-unrelated, mild idiopathic iron overload (possible hereditary hemochromatosis) in Northern Italy.

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In Italian population, typical HFE mutations account for 64% of overt hereditary hemochromatosis (HH)(versus 100% in other countries of Celtic descent).A common HFE-unrelated disease was hypothesized. One thousand and fifty candidate blood donors were screened by iron tests,C282Y and H63D HFE mutations in a region in North Italy. Subjects with repeated fasting transferrin saturation of 45% or more and no secondary iron overload were defined as probands with idiopathic iron overload. To assess the inheritance of iron overload, relatives of probands were screened.
The overall frequency of idiopathic iron overload probands was 3.43% (95% confidence interval, 2.32 to 4.52). 8.4% of them had genotypes associated with HH (compound heterozygous for H63D/C282Y or homozygous for H63D HFE mutations). 91.6% had atypical genotypes: 47.2% were heterozygous for C282Y or H63D HFE mutations, and 44.4% had wild type homozygote genotype.Iron overload was familial in 33.3% of probands with atypical genotypes (1.04% of the overall population). Pedigree analysis excluded linkage of heterozygous HFE mutations with iron overload (cumulative lod score -2.41) and documented a recessive non HLA-linked locus accounting for iron overload in wild type homozygote genotypes. None of the probands had clinical signs of iron accumulation; in males, serum ferritin positively correlated with age ($r=0.63$, $P<0.01$), and the regression model predicted a serum ferritin of 700 ng per millilitre at the age of 58.
Conclusions. In Northern Italy an HFE-unrelated, mild idiopathic iron overload is highly prevalent. A recessive locus accounts for iron overload in at least 1.04% of the overall population.

P0262. Assessing Dental Phenotypes

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More than 5878 gene loci for inherited human diseases and disorders are listed in OMIM of NCBI database with over 1250 related to oral-dental-craniofacial diseases and disorders. Dental defects are seen in numerous of these syndromes. Each dental abnormality or phenotype: number, shape, size, structure, colour, eruption, corresponds to specific genetic and developmental issues. Today more than 200 genes regulating tooth development have been identified (http://bite-it.helsinki.fi). Unfortunately these dental phenotypes are vague and poorly described in the genetic literature. The involvement of a specialised dentist in a genetic diagnostic and counselling clinic provides a dental examination and therefore dental phenotypic data to the genetic team. Systematised data collection and recording of this information are necessary as these observations may relate to rare undiagnosed conditions that might in the near future be revisited and accurately diagnosed. Explaining the oral components of the disease to the patients and their families as well as coordinating appropriate dental referrals according to treatment needs is also important. This service will contribute to the general wellbeing and dental health care of the patient.

P0263. Families with multiple idiopathic impactions of teeth

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We are presenting six families with apparently new genetic disorder characterized by:
1)special unwillingness of the permanent teeth to eruption: eruption is often delayed or incomplete, or the affected teeth remain impacted
2) permanent molars are always affected, other type of teeth less often
3) teeth in infra-occlusion develop ankylosis
4)the disorder is resistant to orthodontic treatment
Less frequently occuring signs are:
5) submersion of deciduous molars
6) delayed eruption of deciduous dentition
7) submersion of some permanent teeth
8) rarely hyperodontia in permanent molar region or
9) transposition of cuspids and bicuspids
Our families could by divided in two groups according to the type of affected teeth and other features, genealogical analysis shows in both groups very probably autosomal dominant type of inheritance. But, the occurence of the disorder in three generations, higher female sex ratio and genealogic data showing no male to male transmission suggest in one group even X-linked dominant inheritance.

P0264. Constitutional mosaicism for a partial trisomy 8 in a patient with a Chromosome Breakage Syndrome

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We report on a male infant with mosaicism for partial trisomy 8 and a generally increased chromosome instability. He was born at term, birth weight 2940 g, head circumference 30 cm (< 3rd percentile). Due to microcephaly, failure to thrive and additional dysmorphic facial features as sloping forehead, retrognathia, low set ears and clinical suspicion of fragile X syndrome the patient was subjected for cytogenetic and DNA analysis at an age of 3 month. There were no genetic disorders or anomalies in family history, except one patient with Down Syndrome. By molecular genetic analysis of FMR1 gene a FRAXA syndrome could be excluded. However cytogenetic results showed a mosaic for a marker chromosome in about 25% of all mitoses and a high number of chromosomal rearrangements, in particular balanced and unbalanced translocations preferentially involving chromosomes 7,8 and 14 with decreasing relative incidence. Multicolour FISH and whole chromosome paint analysis demonstrated that the marker chromosome is derived from chromosome 8 leading to the following karyotype: mos 47,XY,+mar.ish der(8)(wcp8+) [7] / 46,XY [23]. Improved banding analysis performed subsequently allowed to specify a terminal deletion with a breakpoint at 8q21.1 in the aberrant chromosome 8. So far there is no indication for a hematological anomaly or malignancy, but a cytogenetic analysis of fibroblasts was also not performed yet to confirm a constitutional mosaicism. It is interesting that the breakpoint in 8q21 is identical at the cytogenetic level with the Nijmegen Breakage Syndrome (NBS1) gene locus. Therefore further molecular studies will be performed.

P0265. Clinical Description of a New Patient with a de Novo t(6;7;8;12) Karyotype

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We present a 2.5 year old girl with global developmental delay, dysmorphic features and a complex translocation as follows: 46, XX t(6;7;8;12). This child was born at 35 weeks gestation following a complicated twin pregnancy. Her birth weight was on the 10th percentile. She was first seen at the age of eight months presenting with developmental delay, compared to her twin brother, and soft dysmorphic features. Her developmental milestones remained delayed and she also had behaviour disturbances. A.S. has a round face with a prominent metopic ridge, short upslanting palpebral fissures, a mildly depressed nasal bridge and a long philtum. Her twin brother has none of the afore mentioned features. She was thoroughly investigated and cytogenetic analysis revealed a 46,XX t(6;7;8;12) karyotype. This was confirmed by FISH analysis.
Her family was tested and this chromosomal aberration was not found in either her twin or her parents. We therefore present this case of de novo 46,XX t(6;7;8;12) translocation.

P0266. The Macrocephaly-Cutis Marmorata Telangiectatica Congenita Syndrome. Report of six patients and definition of the diagnosis criteria.

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The macrocephaly-cutis marmorata telangiectatica congenita syndrome (M-CMTC) was recently defined as a new overgrowth syndrome characterized, in particular, by specific neurologic and cutaneous conditions. Previously, this disorder was just described as « cutis marmorata telangiectatica congenita syndrome with additional clinical features ». Since then, 39 patients were reported with a high degree of phenotypic variability.
We report 6 additional cases, in particular, we describe the first patient with an arterial dysplasia (Nishimoto desease) and cardiac malformations (atrial septal defect and atrial septal aneurism). Moreover, the analysis of our cases and a review of the literature allowed us to delineate the diagnosis criteria of the M-CMTC.
These data suggest that major diagnostic criteria clearly comprise a neonatal hypotonia, a macrocephaly >>2 S.D., a cutis marmorata, a midline facial naevus flammeus, a hypertelorism, and abnormalities at the cerebral imaging. Minor criteria are represented by a 2,3 syndactyly of the toes, a facial or body hemihypertrophy, some distinctive facial features, a naevus flammeus of the upper lip and /or the philtrum and a hydrocephaly. We propose that the presence of, at least, five of the six major criteria or four major criteria with three minor criteria are necessary for the diagnostic of M-CMTC.

P0267. A balanced 9;18-translocation associated with growth retardation, speech impairment, deep-set eyes and prominent nose.

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We describe a four-year-old boy with growth retardation below the third percentile, hyperactivity, no speech development, deep set eyes, long brushy eyebrows and a broad, prominent nose. The karyotype showed a balanced translocation 46,XY,t(9;18)(q31.2;q21.3). The phenotypically normal father carried the same balanced translocation. High resolution comparative genomic hybridization did not reveal any imbalance in the proband. By fluorescence in situ hybridisation with BAC probes the breakpoint regions have been narrowed to 500 kb on 18q21 and distal to *CAPE* on 9q31.3. Initially, the diagnosis of Floating-Harbor syndrome was considered, which is a rare disorder of unknown etiology characterised by prenatal onset of short stature with delayed bone age, language delay and a triangular face with a prominent nose and deep-set eyes. However, some features including the metacarpo-phalangeal profile and the postnatal onset of the short stature were not characteristic for this syndrome. Although the association between the translocation and the clinical features may be a chance finding, this translocation presents the first potential lead to the identification of chromosomal regions and candidate genes associated with Floating-Harbor like features. This possibility will be tested in a panel of patients with classical Floating-Harbor syndrome.

P0268. The Phenotype of a Boy with a Complex Chromosomal Translocation

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We present a 15 year old boy with dysmorphic features, a seizure disorder, learning difficulties, and a chromosomal constitution of 46,XY,t(2;3;15).
P.D. was born at 37 weeks gestation with a birth weight just below the 50th percentile. He was diagnosed as having an arrested hydrocephalus (a CT scan revealed dilatation of the ventricles) at the age of 6 months. He had frequent urinary tract infections, which were attributed to vesicoureteral reflux (this resolved itself). He had constipation attributed to dolichosigmoid. On examination he was found to be a short young man, with a head circumference above the 95th percentile. He had synophrys, a high nasal bridge, long columella and hypoplastic nostrils. His philtrum was upturning and his chin prominent. P.D. also had rhizomelic shortening of his upper limbs. He was on treatment for seizures. He was thoroughly investigated and found to have a 46,XY,t(2;3;15) karyotype, by giemsa banding and FISH.
His family was also tested, except for his father who is deceased, and were all found to have normal karyotypes.
We believe that this is a de novo translocation in either the father or the son.

P0269. Complete Hydatiforme Moles And Coexistent Viable Foetus. Report of 4 Cases

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Complete hydatiforme mole coexistant with a normal fœtus is a rare obstetric pathology, the incidence reported varying from 1/10000 to 1/100000. It results from fertilization of an empty egg by a hapoid sperm which duplicates without cytokinesis and restores diploidy, or by two sperms.
Outcome depends on maternal criteria of gravity : the risk of persistent trophoblastic disease (PTD) is higher than is single complete mole and seems to be correlated to zygocity mechanism identified by molecular analysis.
We report 4 well-documented cases of complete hydatidiform mole (CHM) coexistent with a twin-live fetus (CHMLF). All of them were spontaneous pregnancies, 2 ended by delivery of a live-born baby, the 2 others were terminated because of maternal pre-eclampsia associated with intra-uterine fetal death.
If cytogenetic studies are sufficient for determining the ploidy, molecular analysis are necessary to confirm the mecanism.
Genomic DNA was extracted from the placenta, molar tissue and peripheral blood leucocytes of parents. Using 10 microsatellites marquers, we showed the paternal origin the molar tissue, and homozygosity of the mole was confirmed in the four cases.
As very rare, more cases are needed to predict the outcome.
Expectant management can be only discussed in absence of maternal complications, associated with a normal fetal karyotype. However, treatment criteria are still to be improved and diligent maternal follow-up is always warranted in post-partum.

P0270. Paternal costitutional mosaicism in familial bpes

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Blepharophimosis-ptosis-epycanthus inversus syndrome (BPES) is an autosomal dominant disorder recently ascribed to mutations in the FOXL2 gene, a forkhead transcription factor. In type I BPES a complex eyelid malformation is associated with premature ovarian failure (POF), whereas in type II BPES the eyelid defect occurs as an isolated entity. Here, we report a family with one child affected by BPES.Both parents were clinically unaffected , and there was no family history of an eyelid defect.
Screening for mutations in the FOXL2 gene in the patient using direct sequencing revealed a frameshift deletion in the coding region, giving rise to a truncating protein.The same mutation was found in both peripheral blood and fibroblasts of the father in mosaicism with a percentage of about 25%. A reexamination of clinical findings in the father revealed, indeed, a minimal reduction of the horizontal diameter of the palpebral fissures.
This study reports the first documented case of costitutional mosaicism in BPES and serves as a reminder that mosaicism should be considered in every case when a mutation is found.
This observation has a significant impact on genetic counseling of sporadic cases of BPES. In case of the paternal mosaicism an accurate evaluation of the mosaicism distribution in the germinal cells could provide an important tool to assign an accurate risk of recurrence in the family.

P0271. 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 form unaffected ones. All carriers and affected patients were diagnosed accurately. Thus this method is effectively usable in prenatal diagnosis of ataxia telangiectasia.

P0272. A new congenital disorder of glycosylation in a girl.

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Congenital disorders of glycosylation (CDG syndromes) are a new group of inherited metabolic disorders. They are characterised by a defect in the synthesis of the glycan chain of the glycoproteins. To date, 8 enzymatic defects are identified. Here we report on a girl with a new type of congenital disorders of glycosylation.
She was the third child from healthy consanguineous Tunisian parents, born at 35 weeks gestation after an uneventful pregnancy by caesarean section. Birth weight was 2440 g (-1 SD), length 49 cm (+1 SD), and head circumference 33 cm (+1 SD). Poor sucking, generalised hypotonia, facial dysmorphism and hypocalcemia marked neonatal period. Cardiac and transfontanellar ultrasounds and blood chromosome with 22q11 in situ hybridisation were normal. At 6 months she had failure to thrive, feeding difficulties requiring tube feeding and gastrostomy, severe psychomotor involvement, major hypotonia, progressive microcephaly and frequent ORL and respiratory infections. At 18 months, she was not able to sit and speech. Cerebral MRI was normal. Coagulation factors, serum cholesterol level and hepatic function were normal. Metabolic investigations were normal. Immunologic investigations showed a low IgG level with normal IgA, IgM . Iso electric focusing and western blot analysis showed a mild hypoglycosylation of proteins but phosphomannomutase and phophomannose isomerase activities were normal, excluding CDG Ia and Ib. The patient's fibroblasts showed a incompletely assembled dolichol linked oligosaccharides with 7 mannose residues secondary to an inefficient addition of the last mannose containing branch that is required for protein N-glycosylation. Molecular biology investigations are in progress.

P0273. Spectral karyotyping study of three patients with constitutional supernumerary chromosomal markers.

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Currently, the GTG-banding technique is the most commonly used diagnostic method in clinical cytogenetics. This analysis of markers can be inadequate because of poor chromosome morphology, size of markers and/or an insufficient yield of analyzable metaphases. The usefulness of the FISH remains limited by the number of spectrally distinguishable fluorochromes or fluorochrome combinations.
Spectral karyotyping (SKY) has been developed to unambiguously display and identify all chromosomes at one time using a spectral signature that generates 24 unique colors. Three cases of constitutional supernumerary chromosomal markers and different clinical manifestations are described in this report. Case 1: a male (9 months of age) with microcephaly, up-slanting palpebral fissures, malformed ears, cryptorchidism, and “schawl” scrotum. Case 2: a 6-year-old female with facial asymmetry, strabismus, prominent nasal bridge, micrognathia, and cubitus valgus. Case 3: a 14-year-old male with seizures, triangular face, strabismus, prominent ears, thoracic asymmetry, scoliosis, cubitus valgus, macro-orchidism, and wide gap between first and second toes. SKY reveals the origin of the three markers (Cases 1, 2, 3) as der(2), der(9), and der(15), respectively. The identification of the chromosomal origin of the markers by SKY provides better information for the physician, and genetic counselor, for more appropriate management of patients with specific aneusomies.
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P0274. CDG syndome type 1 : Ovarian histopathologic anomalies in a woman with hypergonadotrophic hypogonadism

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Carbohydrate Deficient Glycoprotein (CDG) syndrome type 1 is a genetic disorder with multisystemic manifestations. Few affected adults have been reported and most females show absent or delayed pubertal development due to ovarian failure but no histopathological examination in adult patients has been reported to date.
We present the case of a 22-year-old woman, with CDG syndrome type 1. At age of 17 she had no sign of pubertal development. No ovaries were present on ultrasound examination and endocrine testing revealed hypergonadotrophic hypogonadism.
Pelvic laparoscopy showed bilateral streak-like ovaries with dysmorphic fallopian tubes and a normal uterus. Biopsy specimen of ovaries revealed ovarian like stroma with no follicle.
Less than 10 adult women with CDG syndrome have been reported. Only one woman had normal puberty. All the others present with hypergonadotrophic hypogonadism. In half of the cases no ovary is detectable by ultrasound examination, in the other half of cases ultrasound revealed presence of ovaries but without signs of follicular activity.
Abnormal protein glycosylation has complex and heterogeneous effects which result in ovarian failure. Details of pathogenesis remain to be determined.

P0275. An autosomal recessive cerebello-oculo-renal syndrome unlinked to both the JBTS1 and NPH1 loci

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A delineation of several syndroms featuring cerebellar vermis hypoplasia, and ocular, renal, or hepatic involvement has proven difficult. Cerebellar vermis hypoplasia occurs as an isolated trait, or together with hypotonia, developmental delay, and abnormal breathing or abnormal eye movements in Joubert syndrome, and is further observed associated with other ocular, renal, or hepatic involvement. These combinations including Arima, Senior-Löken and COACH syndromes, have been considered by some authors as variants of Joubert syndrome and as distinct disease entities by others. In addition, cerebellar vermis hypoplasia occurs together with juvenile nephronophthisis. We report a large consanguineous Austrian family with three children, two sibs and their cousin, being variably affected by cerebellar, ocular, and renal malformations. We present a comparison of the clinical findings with known cerebello-oculo-renal syndromes, and the exclusion of both the Joubert syndrome and juvenile nephronophthisis loci, JBTS1 and NPH1, respectively, as the site of the mutant gene.

P0276. Computer assisted diagnosis of chromosomal aberrations using a Bayesian and a counting approach with the help of the database SYNDROC

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The possibility to diagnose chromosomal aberrations using a computerized database was tested using 101 patients with an established chromosomal aberration using the database SYNDROC. This system provides the user with two different algorithms for the calculation of a diagnosis:
- a descriptive algorithm which proposes a diagnosis counting a set of phenotypic markers all having the same weight.
- a Bayesian-algorithm which, evaluating calculates probabilities for competing diagnoses by analyzing phenotypic anomalies.
Three levels of precision were used assessing the diagnoses: suggestion of the correct (1) chromosome number, (2) chromosome arm, (3) aberration type and rough location.
The combination of both algorithms yielded 51 consensus diagnoses for the level of the correct chromosome, 24 for the chromosome arm, and 15 for the aberration type. Additional diagnoses solely with the descriptive algorithm were yielded for 43, 52 and 47 cases and using the Bayesian-algorithm for 1, 4 and 1 diagnoses respectively. Since with the Bayesian-algorithm, when evaluating an uncertain diagnosis using a combination of symptoms, one does so by calculating the probability of the claim in the light of given information. This seems to be a much more promising for a correct diagnosis than the pure counting of numbers of matches. The Bayesian coefficients were in the range between 0.57 and 0.05; the 0.5 margin as a trustworthy one announced by the authors of SYNDROC was reached by only one of the correctly recognized cases. The prior probabilities for the calculations of the Bayesian-formula do not seem to use serviceable weights.

P0277. Another observation with VATER and a deficiency of complex IV respiratory chain disorder.

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The VATER association of vertebral anomalies (V), anal atresia (A), esophageal atresia and/or tracheo-esophageal fistula (TE), radial and renal anomalies (R) is a common congenital disorder of unknown origin with probably heterogeneous causes. Here, we report on a girl presenting with pre- and postnatal growth retardation, esophageal atresia, vertebral and costal anomalies and a unilateral radial defect, consistent with the diagnosis of VATER association. In the first months of life, she presented with failure to thrive, severe episodes of hypoglycemia, and liver cytolysis which prompted us to perform a metabolic screening. Hyperlactatemia was observed and a complex IV respiratory chain deficiency was found on a liver biopsy. The respiratory chain deficiency was not observed in skin fibroblasts. No mtDNA point mutation or deletion was identified. The girl is now 6 years old and has a normal mental development but persistent feeding difficulties and moderate hyperlactatemia (2.6mM). To our knowledge, this is the second report of VATER association with a mitochondrial disorder. In a previous report (Damian et al., 1996), a VACTERL association was observed in a girl who presented, in addition, the mitochondrial NP3243 point mutation. The observation

of VATER association in combination with a mitochondrial disorder may be coincidental but could suggest also that the presence of multiple malformations and antenatal manifestations does not rule out the diagnosis of respiratory chain deficiency.

P0278. Nager syndrome: About a Tunisian case

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Nager acrofacial dysostosis was recognized as a specific entity by Nager and de Reynier syndrome(1948). It's a rare desorder, approximately 40 documented cases. The inheritance is autosomal dominant, most cases have been sporadic. Zori(1993) suggested that the gene may reside on chromosome 9.
We reported a young boy offspring of young and non consanguineous parents, he presented a mandibulofacial dysostosis and a skeletal anomalies, evoking Nager's syndrome.The investigates genetics is negative. We begin in this work a comparative study with the data of the literature and we discuss the differential diagnosis.

P0279. Long follow up in a patient with a Toriello-Carey syndrome.

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In 1988, Toriello and Carey described a rare congenital disorder characterized by telecanthus, short palpebral fissures, a small nose, abnormal ears, Robin sequence cardiac defect, corpus callosum agenesis, hypotonia, postnatal growth retardation and developmental delay. Originally an autosomal recessive mode of inheritance was suspected. Since that times a total of fourteen patients have been reported. The predominance of affected male and the milder phenotype in female patients suggested an X linked or sex influenced gene. We report the follow up of an additional male patient with a Toriello-Carey syndrome born from unconsanguineous parents from birth to the age of 7 years.

P0280. Ocular Findings In Fabry Disease : A Survey Of 25 Hemizygous Male Patients

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Background: Fabry disease (FD) is an X-linked inborn error of glycosphingolipid metabolism due to deficient activity of lysosomal α-galactosidase A. Progressive glycosphingolipid storage is responsible for renal failure and ischemic complications, involving brain and heart. Much interest is currently paid to emerging α-galactosidase A replacement therapy.
Methods: We carried out a complete baseline ophthalmologic examination of 25 hemizygotes affected with FD, prior to enzyme replacement therapy.
Results: The mean age at time of examination was 36 ± 13 years. No patient had any functional complain. The measured refractive values were unremarkable. However, the incidence of myopia was high (46.00 %). Thirty-nine eyes (78.00 %) reached a far best corrected visual acuity (BCVA) of 20/20. Seven eyes had a BCVA of 20/25 and none of them had an acuity lower than 20/33. All the eyes had normal BCVA in near vision. The mean value of the Schirmer 2 test was normal in 18 patients. Seven patients (28.00 %) presented a reduction of the lacrimal secretion. Vascular abnormalities of the bulbar conjunctiva were found in 34 eyes (68.00 %). *Cornea verticillata* was observed in 23 eyes (46.00%). A corneal haze was the most frequent corneal abnormality, observed in 45 eyes (90.00 %). Lens anterior capsule deposits were observed in 4 patients and the Fabry posterior cataract in 9 patients. Retinal vascular tortuosities were observed in 12 patients (48 %). The optic discs were unremarkable. An enlargement of the blind spot was noted in 35.00 % of the eyes at visual field examination.

P0281. Constitutional Microdeletion del(22)(q12.1q12.3) including the NF2 gene region.

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A de novo deletion of the long arm of chromosome 22 distal to the DiGeorge/Catch22 critical region as verified by FISH analysis in a 7 1/2 year old girl is described. This type of cytogenetically visible microdeletion was not reported so far. The patient was born as the second child to healthy unrelated parents following an uneventful pregnancy. Her phenotypic anomalies are relatively mild including a moderate growth and mental retardation and discrete dysmorphic facial features can be seen as well. Initial cytogenetic results obtained by standard G-banding were confirmed by high resolution RBG/GBG banding and characterized in more detail by analysis of the patient and her parents using more than 8 informative microsatellite markers including D22S536, D22S1167, D22S273, D22S278 and D22S1156. The patient does not show cafe-au-lait spots or neurofibromas at her skin however since the gene for neurofibromatosis 2 coding for the merlin/Schwannomin tumor suppressor protein maps to 22q12.2, which is by cytogenetic resolution within the deleted segment, further molecular studies are required to provide the patient with the optimal disease prevention and medical management.

P0282. Mutations in *EDA, EDAR, XEDAR* and *NEMO* genes reveal a new signal transduction pathway participating in differentiation of skin appendages.

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Anhidrotic ectodermal dysplasia (EDA) is caused by the defect in the differentiation of skin appendages during embryonic development, resulting from improper interactions between ectoderm and mesenchyme at a molecular level. Initiation of the differentiation process requires protein products of *EDA, XEDAR* and *NEMO* genes localised on the X chromosome and *EDAR* gene localised on chromosome 2. These genes encode a ligand (ectodysplasin A), receptors (EDAR and XEDAR) belonging to TNF/TNFR families, as well as a protein (NEMO), participating in signal transducting pathway involving NFκB. Patients harbouring mutations of these genes exhibit an identical phenotype: oligodontia or anodontia, sparse hair and hypertermia, caused by the lack of sweat glands.
The structure of *EDAR* and *XEDAR* genes was investigated in 20 patients with clinical symptoms of anhidrotic ectodermal dysplasia and their 80 relatives. In these patients no mutations in *EDA,* and *NEMO* genes were found. Appropriate fragments of genomic DNA were amplified by PCR and were subjected to multiple temperature, single stranded conformation polymorphism analysis (MTSSCP) followed by direct sequencing using automated sequencer. In one patient, sequence analysis revealed a novel T1109C transition resulting in (Val370Ala) substitution in the death domain of EDAR. In the other patient, sequence analysis demonstrated deletion of G (del252G) in exon 2 of *XEDAR* gene resulting in premature termination of translation and truncated form of the receptor devoid of transmembrane and intracellular domains. The correlation between the phenotype and the localisation of the molecular defect was investigated.

P0283. Diagnosis of malformation syndromes using artificial neural networks

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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 was applied, 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 numbers of symptoms/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.

P0284. Can OMIM be used as a decision support system in the diagnotic process of malformation syndromes?

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The increasing number of malformation syndromes causes difficulties for the diagnostic process of the clinician. OMIM (Online Mendelian Inheritance in Man) was tested as a decision support system for the differential diagnostic process of patients with malformations. The data of 119 patients with different clinically confirmed syndromes were used to search differential diagnoses in OMIM feeding the signs of the respective patient in all possible combinations. Four different search strategies were tested (1) utilization of all signs of the patient, (2) usage of those signs, that a clinical expert considered to be important in diagnosis, (3) input of head-neck-signs (4) feeding all but (3). The combinations of signs for the searches in OMIM were created using the 'AND' and the 'OR'-junction.
With the AND-junction the number of differential diagnoses decreased with the number of signs used in combination, while the OR-junction resulted in more than 200 differential diagnoses for every syndrome and search strategy on the average. All four search strategies yielded more than 50% positive results, interestingly even strategy (4) was very effective. The number of differential diagnoses decreased with the number of signs read in in combinations applying the AND-junction.
The application of OMIM is sometimes tedious, but it is an acceptable and useful tool in all four strategies although it has not been developed as a decision support system in the realm of malformation syndromes.

P0285. Stuve –Wiedemann syndrome in long-term survivors: a neuro-myo-skeletal disorder with prominent neurovegetative features

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In 1971 Stuve and Wiedemann described a syndrome characterized by bowing of long bones, camptodactyly, respiratory distress, hypertermic episodes and death in the first year of life (Z Kinderheilkd 11:184-92,1971). Clinical features of long-survivor patients with SWS are not studied in great detail, owing to the rarity of individuals.
We follow two patients with SWS aged 12 and 3 years. The first had congenital bowing of the long bones, respiratory distress, swallowing difficulties and unexplained fevers in the first year of life. After, he developed intolerance to low temperature, paradoxical sweeting, hypolacrimation and lack of corneal reflex leading to keratitis and corneal leukomas, recurrent ulcerations of the tongue, chronic gingivitis and dental decay. Cognitive level is normal. He has no clinical signs of myopathy, but muscle examination showed an increased number of lipid droplets and reduced respiratory chain activities. No mtDNA mutations were detected. After the first years of life he also showed severe progressive scoliosis. The second patient had respiratory distress, hypertermia, bowing of legs and camptodactyly at the birth. In the second year of age he developed intolerance to low temperature, paradoxical sweeting, tongue ulcerations with loss of tongue fragments, poor dentition, chronic gingivitis, corneal anesthesia leading to keratitis and corneal leukomas. Cognitive level, electromyography and motor nerve conduction are normal.
Clinical history of our patients expands the clinical phenotype of this intriguing disorder and may help in pinpointing candidate genes.

P 4. Cystic fibrosis and Familial Mediterranean Fever

P0286. Maternal UPD7 In The Case Of Cystic Fibrosis

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Uniparental Disomy (UPD) is the inheritance of two homologous chromosomes only from one parent in a euploid offspring. Loss of heterozygosity in the uniparental isodisomy may be a cause of clinical expression of autosomally recessive disorders, when only one of the parents is the carrier of gene mutation. In approximately 7-10% patients with Silver-Russell syndrome maternal UPD7 is observed. Clinical, cytogenetic and molecular studies carried out in the case of a 2 years old girl affected with cystic fibrosis (CF) as a result of maternal isodisomy of chromosome 7 are presented. Clinical diagnosis of CF was confirmed by mutation analysis in the CFTR gene which revealed that patient is the homozygote of delF508 mutation. Severe intrauterine and postnatal growth retardation (body weight -4SD, body length -6SD), relative macrocephaly, clinodactyly of 5th finger, feeding difficulties, hypoglycaemic episodes in the neonatal period and dysmorphic features suggested Silver-Russell syndrome. Psychomotor development was within the normal range. Karyotype in the lymphocytes was normal - 46,XX. Analysis of polymorphic microsatellites markers at loci: D7S507, D7S2422, D7S460, D7S1517 and GTNOS showed maternal UPD7. Analysis of delF508 mutation revealed that only mother was the heterozygote of this mutation.
Results of our studies explain the cause of severe somatic retardation in the patient.The diagnosis was the basis for verified genetic counselling concerning recurrence risk of cystic fibrosis in that family.

P0287. The case of cystic fibrosis in 46,XY phenotypic newborn girl with Smith-Lemli-Opitz syndrome

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In presented study we are describing a phenotype of newborn girl consulted concerning congenital abnormalities. Her mother was 20 years old, father was 25 years old, apparently healthy. The parental consanguinity wasn't discovered. Child was born from fifth pregnancy, complicated with toxemia, at 38 week of gestation, birth weight was equal to 2700g, head circumference of 33 cm. The first four pregnancies terminated with spontaneous abortion. There were the following phenotypic abnormalities: broad nasal bridge with broad nose and anteverted nostrils epicanthal folds, low set ears, increased nasolabial distance, broad maxillary alveolar ridges, micrognatia, cleft palate, short neck with pterygium colli, postaxial polydactyly of hands and feet, hypoplastic of thumbs, proximal cutaneous syndactyly of toes II-III, congenital heart defect, external genitals feminine. The karyotype of child was 46,XY. The karyotypes of both parents were normal. The child died at the age of 15 days. It was found the following malformations at autopsy: microghiry, heart trilocalar with common ductus arteriosus, fibrosis interstitial of pancreas with dilatation cystic of sinuses, cystic dysplastic changes of both kidneys, bronchitis and broncholith with mucus obstruction. Thus we suggest that above mentioned case is presented by association of Smith-Lemli-Opitz syndrome in phenotypic female a 46,XY karyotype and cystic fibrosis caused by different recessive genes.

P0288. Prenatal Diagnosis in Families with Cystic Fibrosis in Republic of Moldova

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Introduction: Taken into consideration that there are 72% of mutant chromosomes with non-identified mutations in moldavian patients with Cystic Fibrosis (CF), we performed prenatal diagnosis (PD) using both molecular-genetic and biochemical methods. Using PCR-RFLP analysis and investigation of the level of the some enzymes in liquor amnii (aminopeptidaze, γ-glutamiltranspeptidaze and alkaline fosfotaze) have been applied PD in 12 CF-families.
Results: In all 5 cases of PD in first trimester of gestation we used only molecular-genetic methods. In 2 cases we based only on the

availability of the DF508; in 1 case - on the availability of DF508 and R347P; in 2 cases - on the availability of DF508 and allelic distribution in CS7/Hin6.I and Km19/PstI systems (in both allele 2 was linked with unknown mutations). In all of these cases were established healthy fetuses. In 4 (from 7) cases of PD in the second trimester of gestation we used both the molecular-genetic (availability of DF508) and biochemical methods. Due to absence of information about "guilty" mutations in 3 CF-families we carried out the PD in the second trimester of gestation based only on the data of the enzyme's level in liquor amnii. As a result of our PD investigations we confirmed cystic fibrosis in 4 fetuses.
Conclusion: Using both molecular-genetic and biochemical approaches of investigation allows performing of PD in total number of pregnancies with major risc in CF in situation when there are not sufficiently information about molecular-genetic structure of mutations.

P0289. Preimplantation Genetic Diagnosis for cystic fibrosis in populations with molecular heterogeneity, based on multiplex sequence variation detection throughout the CFTR gene

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Cystic fibrosis (CF) is targeted as one of the priority genetic diseases for prevention programmes. Preimplantation Genetic Diagnosis (PGD) represents an alternative approach to prenatal diagnosis, especially for couples with an unsuccessful reproductive history and/or undergoing assisted reproduction for male infertility who have the additional risk of transmitting CF. The clinical application requires optimization of single cell genotyping protocols to minimize PCR failure, allelic drop out (ADO) and contamination. In addition the protocol should allow detection of the wide spectrum of potential CF-affected genotypes, especially relevant for Southern European populations. To this end we developed a flexible multiplex PCR protocol allowing analysis of sequence variations in any combination amongst 7 CFTR gene exons (4, 10, 11, 13 in two parts, 14b, 17b and 21) by nested-PCR and DGGE analysis, along with the intragenic dinucleotide microsatellite IVS8CA. The experiments were carried out on 390 single lymphocytes from 3 compound heterozygous CF patients, one heterozygote and one non-CF individual. PCR efficiency between exons varied from 90% to 100%, and ADO from 0% to 3.8%. IVS8CA was co-amplified with PCR efficiency of 92.4% and 10.8% ADO (evaluated by sizing the fluorescent PCR product). No contamination was observed in any set of experiments. The present method overcomes the need of separate assays for each CF mutation, and additionally allows analysis of linked polymorphic sequence variations (when informative), useful for minimizing misdiagnosis and/or indirect diagnosis. This method proved robust and flexible for diagnosing diverse CF genotype combinations in single cells.

P0290. Preimplantation genetic diagnosis (PGD) of cystic fibrosis by multiplex PCR combining deltaF508 mutation and intragenic microsatellites of the CFTR gene.

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One major limitation of pre-implantation genetic diagnosis (PGD) practice comes from the need to develop single cell PCR protocols. For Cystic fibrosis (CF), for which almost 1000 mutations have been identified, the development of a mutation-based PGD protocol is impracticable. An way to overcome this problem is to set up an indirect diagnosis using polymorphic markers allowing the identification of the pathogenic haplotype instead of the mutation. We present a new PGD protocol for CF, based on a multiplex fluorescent PCR co-amplifying the deltaF508 mutation and two CFTR intragenic polymorphic microsatellites (IVS8CA and IVS17bCA). Such an approach is justified since in 91% of the cases at least one partner of the couple carries the deltaF508 mutation. The use of intragenic markers reduces the risk of misdiagnosis due to meiotic recombination. A PCR signal was obtained in 97% of the single lymphoblasts (151/155) tested. A complete haplotyping was achieved in 137/151 (91%) lymphoblasts and a 6% rate of allele drop-out (ADO) was observed. During clinical application, 94% of blastomeres gave PCR signals and a complete haplotype could be assigned to 84% of them. With the degree of polymorphism of the markers (48 and 39%) and their co-amplification with the F508 locus, our test should be suitable for nearly 80% of the couples requesting PGD for CF. This fluorescent multiplex PCR indirect diagnosis provides also a safer test since it allows confirmation of the diagnosis, detection of contamination and could give an indication on the ploidy of the embryos tested.

P0291. Non-visualization of the fetal gallbladder: a predictive sign of cystic fibrosis?

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The non-visualization of the gallbladder without additional malformations has been described in fetuses with trisomy 21, biliary atresia and gallbladder agenesia. On another hand, a number of fetuses with an isolated absence of the gallbladder have a normal outcome. It is well known, however, that cystic fibrosis must be suspected when the absence of the gallbladder is associated with hyperechogenic bowel. We report here 5 cases where the isolated non-visualization of the gallbladder led us to diagnose cystic fibrosis by DNA analysis at 20-24 weeks of gestations. Four fetuses were homozygous ΔF508/ΔF508 and 1 was coumpound heterozygote ΔF508/G551D. All pregnancies were terminated and the gallbladder was found to be present but hypoplastic on pathological examination in all 5 fetuses. Several hypothese may be proposed to explain this finding. 1) a cystic duct obstruction from inspissated bile or mucus. Pathological examinations of the biliary tract were however normal in all fetuses.2) a contracted gallbladder. 3) an hyperechogenic bowel might have been associated earlier in pregancy and might have disappeared when ultrasound examinations were performed. In agreement with this hypothesis, one of the fetuses reported her was found to have an absent gallbladder associated with hyperechogenic bowel at 14 weeks of gestation whereas the anomaly of the gallbladder was isolated at 15 weeks of gestation. We suggest therefore to performe chromosomal and DNA analyses on amniotic fluid to exclude trisomy 21 and cystic fibrosis respectiviely when the gallbladder is not visualized at 22 weeks of gestation.

P0292. Non-invasive Prenatal Detection Of A Paternal Inherited Cystic Fibrosis Mutation In Maternal Plasma

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The discovery of the presence of circulating fetal DNA in maternal plasma and serum opens up new strategies to perform non-invasive procedures to obtain a reliable diagnosis without any risk for the fetus. In this way, it has been used for non-invasive prenatal diagnosis of fetal gender, Rh factor, and paternally inherited disorders like myotonic dystrophy.
The big potential of the detection of fetal DNA from maternal plasma by PCR lies in the possibility to avoid any risk for the fetus in the invasive procedure and its feasibility for a clinical application. Because of the maternal DNA contamination this kind of analysis is limited to those sequences of paternal origin and thus we attempted to detect only the paternal mutation.
In our study we have used the PCR method to demonstrate a non-invasive prenatal analysis, in maternal plasma, of an autosomal-recessive disorder like Cystic Fibrosis in which both parents have different mutations, having successfully detected a paternally inherited CF mutation in heterozygosis.
The interference of the maternal DNA during the PCR amplification represents the main disadvantage. At this point it is essential to find new strategies in order to obtain a non-invasive diagnosis with results as precise as the ones obtained by invasive procedures.

P0293. Microscopic fetal pathological examination can lead to unexpected diagnosis of cystic fibrosis

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We are reporting a case of a 19 weeks gestational age fetus terminated for myelomeningocele discovered on early ultrasound examination . Parents were non related with no story of familial genetic disease. On microscopic examination of fetal pancreas, slight cystic dilatations of small pancreatic ducts were consistent with cystic fibrosis as these dilatations are known as the earliest histologic change described in the pancreas. Molecular analysis of the CFTR gene by DHPLC on the genomic DNA of the parents revealed the presence of the mutations deltaF508 and E60X, thus confirming this diagnosis.
Normal pancreatic development is caracterised by the increase of acinar number during pregnancy. In case of cystic fibrosis, acini become scarce with extensive diffuse fibrosis and excretory ducts may be distented with eosinophilic plugs in their lumina. Others additional findings can be meconium ileus, or meconial peritonitis and on microscopic view widespread obstruction of exocrine glands ducts. However none of these morphological findings can be found in every fetal or perinatal case but becomes usual in older children.
To conclude, this case report points out the need of thorough fetal examination which can discover others abnormalities than the expected ones and allows a better pre-natal genetic counselling.

P0294. Screening for CFTR Gene Mutations and Polymorphisms in Patients with Chronic Pancreatitis

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Chronic pancreatitis is an inflammatory disorder in which progressive and irreversible structural changes of the pancreas result in a permanent impairment of both exocrine and endocrine function. Both environmental and genetic factors are known to induce chronic pancreatitis. Recent reports have revealed genetic basis of chronic pancreatitis showing that mutations in Cationic Trypsinogen, CFTR and SPINK1 genes are associated with this disorder.
This study was undertaken to investigate the association of mutations and polymorphisms in CFTR gene with chronic pancreatitis in Yugoslav patients. Thirty-nine patients (alcohol-related pancreatitis in 29 and idiopathic pancreatitis in 10) were examined using the combination of PCR and subsequent direct (HA, PSM) and indirect (SSCP, DGGE) mutation detection methods.
F508del mutation was found in two patients (5.1%). 5T allele at Tn polymorphic site was not found in any of the analyzed patients, while frequencies of 7T and 9T allele were 85.9% and 14.1%, respectively. Eighteen patients (46.1%) had 2694T→G polymorphism in exon 14a, while three (7.7%) had 4002A→G polymorphism in exon 20. One patient showed also, in combination with 2694T→G polymorphism, the change in exon 6a, but this change should be defined through sequencing.
Obtained frequency of 2694T→G polymorphism in Yugoslav patients with chronic pancreatitis (25.6%) closely resembles its frequency in healthy Yugoslav population (29%). Still, obtained frequency of CFTR mutations is slightly increased in these patients which indicates possible association of mutations in CFTR gene with chronic pancreatitis, but further investigations on larger cohort of patients are required in order to confirm these findings.

P0295. Molecular epidemiology of cystic fibrosis in Brittany, France: a retrospective study from 1960

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Cystic fibrosis (CF) is the most common severe autosomal recessive disease that affects children in Caucasian populations. Characterized by pulmonary and digestive disorders, CF is caused by mutations in the CFTR gene. Near 1000 mutations have been identified worldwide. The aim of this study was to define the spatial and temporal distribution of CF and of its mutations in Brittany (western France) where the disease is frequent.
We retrospectively registered all the patients born in Brittany since 1960, by crosschecking different data sources. We contacted councils to obtain patients' residence place.
A total of 520 patients were registered. We assessed CF incidence according to administrative and ecclesiastic divisions and its evolution over decades. Incidence was 1/2630, with a west/east gradient which was confirmed over time (Finistère: 1/2071 vs. Ille-et-Vilaine: 1/3286). This high frequency may result from founder effects and genetic drift. Currently, incidence is decreasing mainly because of prenatal diagnosis. Moreover, we determined the mutations spectrum and their spatial distribution. We obtained an excellent detection rate (99.7%). Western Brittany presented a specific spectrum (1078delT, G551D, W846X, 4005+1G>A), whereas the eastern part show a spectrum more similar to the French one.
This study relates the regional specificities of the CFTR gene and highlights the disparities that existed in Brittany. This results from different isolation degrees and population movements. It is the first time that a so detailed study is performed in a large population. This better knowledge of CF epidemiology allows to improve diagnostic strategies and to refine genetic counselling.

P0296. Notification of Cystic Fibrosis as primary cause of death in Rio de Janeiro State, Brazil, from 1979 to 1998

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Cystic Fibrosis (CF) is an autossomal recessive disease mapped to 7q31-q32. Lung disease accounts for approximately 95% of its morbidity and mortality, with an incidence of 1 in 2,500 Caucasians. The aim of this work was to investigate the notification of CF as primary cause of death in Rio de Janeiro State (RJ) for 20 years. Our data were extracted from the Brazilian Data-SUS CD-rom entitled "Sistema de informacão sobre mortalidade/1979-1998". Our results show that 27 infants, younger than 1 year, died from CF in RJ from 1979 to 1988, and 13 infants died from CF from 1989 to 1998. The same sorts of results were observed throughout Brazil (252 cases from 1979 to 1988 and 178 cases from 1989 to 1998). For Brazil, Qui-square test indicates that these differences are significant ($p<0.01$), as well as for Rio de Janeiro State ($p<0.05$). For the age group 15 to 24 years the numbers of death notifications have increased. These results indicate that there was an improvement in the quality of life of CF patients. Eighty people out of 93 died from CF in Rio de Janeiro City and 13 deaths occurred in five smaller cities, although their permanent addresses were distributed among 12 different cities out of 31. It is worth mentioning that notification of CF as primary cause of death occurred in cities, but one, where there was at least one Faculty of Medicine. This may account for a better acknowledgement of CF and for a more accurate death notification.

P0297. Molecular-genetic analysis of polymorphic variants of GSTM1, CYP1A1 genes and CFTR gene mutations in patients with chronic pulmonary diseases from Bashkortostan

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Chronic pulmonary diseases are one of the major widespread causes of illness. The search for pulmonary disease susceptibility genes is a complex problem, connecting with the influence of environmental factors in the pathogenesis of these disorders. Nevertheless, a large number of epidemiological studies suggest that genetic factors play a role. One possibility to account for a susceptibility to the effects of environmental pollutants may be genetic variations in the xenobiotic metabolizing enzymes such as glutathione-S-transferase M1 (GSTM1) and cytochrome P450 1A1 (CYP1A1). In addition, heterozygosity for predominant delF508 mutation of CFTR gene is associated with pulmonary disease such as chronic bronchitis and asthma.
In order to determine the possible role of detoxifying enzymes gene mutations and mutation delF508 of CFTR gene in the pathogenesis

of pulmonary diseases we have studied 45 patients (22 with bronchopneumonia, 12 with chronic bronchitis, 7 with asthma and per 2 with bronchiectasis and bronchoobstructive syndrome) and compared the results with 68 subjects from control group. The GSTM1 0/0 genotype was found in 37% of affected individuals and in 49% in control subjects. There were no significant differences (p>0,05). For CYP1A1 locus the Val allele was detected in 7% of affected individuals and only in 3% in control, but the differences are not significant (p>0,05). CYP1A1 Ile/Val genotype increased approximately twofold in affected individuals (15%). We have not found an individual with delF508 mutation of CFTR gene. In future studies of multiple gene polymorphisms we should include other candidate genes and will take more affected patients.

P0298. Possibilities and barriers in the implementation of a preconceptional screening programme for cystic fibrosis carriers: a focus group study

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Background. Since the identification of the cystic fibrosis (CF) gene it has become possible to perform CF carrier screening. Despite the positive results of pilot studies in various countries, carrier screening is not yet standard practice. The question arises as to whether this might be due to barriers in implementation.

Objective. The objective was to explore possibilities and barriers in the implementation of a nation-wide preconceptional CF carrier screening programme.

Methods. Sessions with two focus groups of CF patients and CF relatives, one focus group of people from the target population (couples planning to have children), and two focus groups of care-providers (general practitioners, and health care workers in the Municipal Health Services).

Results. In general, there is a positive attitude among CF patients and their relatives, the target population, and care-providers towards preconceptional CF carrier screening. The most important barriers in implementation are the problem of reaching the target population, the heavy workload of GPs, the limited knowledge about CF in general, and the absence of a preconceptional consultation setting.

Conclusion. Different intervention strategies will be necessary to overcome the various barriers in the organisation and execution of the screening. The positive attitude towards preconceptional CF carrier screening, in combination with the willingness of the care-providers to participate in providing (a part of) the screening programme, will make it easier to overcome the barriers.

P0299. Evaluation of the probe specificity used in INNO-LiPA CFTR: No false positives or cross-reactions with benign variants or rare mutations.

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INNO-LiPA CFTR12 and INNO-LiPA CFTR17 + Tn respectively identify 12 and 17 CF-related mutations and their wild-type sequences, as well as the CBAVD-related polymorphism Tn. The technology used is a simple reverse hybridisation of amplified product on a nitro-cellulose strip carrying specific oligonucleotide probes as parallel lines. The amplified product is the result of an optimised multiplex amplification. Hybridisation and stringent wash occur at the same temperature and can be performed either manually or using Auto-LiPA. Both assays were successfully validated in a European multicenter study.

Individuals, homozygous for a mutant or a wild-type allele, will only hybridise with the corresponding probe, whereas individuals heterozygous for a particular mutation will hybridise with both the mutant and wild type probe for this mutation. We undertook to test the INNO-LiPA CFTR assay specificity for samples containing benign variants or rare CF-related mutations, of which the sequences are, covered by the INNO-LiPA CFTR mutant and wild type probes. The benign sequence variants were introduced by mutagenesis and controlled by sequence analysis. In addition, three clinical samples with proven presence of F508C and linked with the S1251N mutation were included. For the rare mutations, clinical samples were available. None of the benign variant sequences tested showed any false-positive reaction with the corresponding mutant probe on the strip; in all these cases, the wild-type probe remained positive. For the rare mutations, no cross-reactivity with the mutant probes was observed. We could conclude that in all these samples, the INNO-LiPA probe reactivities allowed correct clinical interpretation.

P0300. Prevalence Of Cftr Mutations In Newborns With Increased Irt Detected Through A Pilot Neonatal Screening For Cystic Fibrosis In The Piemonte Region

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Neonatal screening strategy. A two-step protocol combining the assay for immunoreactive trypsinogen (IRT) with the analysis of 30 mutations in the CFTR gene using the OLA-PCR-SCS. Neonates with two mutations are referred directly for clinical assessment and confirmatory sweat test; infants with one mutation are recalled for sweat test at age 4-5 weeks. A genetic counselling benefit is offered to parents when trypsinogen/DNA screening is performed. This strategy results in early and accurate diagnosis of cystic fibrosis but an excess of heterozygotes among neonates with hypertrypsinaemia has been reported.

Prevalence of heterozygosity in hypertrypsinaemic newborns. We have assessed the heterozygosity frequency among 50.956 children born from July 2000 to December 2001 and screened for CF. 996 (1.9%, i.e. 1/51) of those tested had an IRT level greater than the decisional threshold and were analysed for mutations. 19 were CF with a positive sweat test and 80 were carriers. Incidence of CF was 1/2682, leading to a carrier frequency of 1/26 while the estimated frequency of heterozygtes in children with hypertrypsinaemia was 1/9, three times greater than the general population. The number of different mutations in carriers is greater than in CF children (13 versus 10) and than in a cohort of 1574 newborns from our region, not selected by IRT, analysed in a previous pilot screening (10 different mutations). The identification of an excess of heterozygotes in hypertrypsinaemic newborns remains an important matter that can be managed only with effective counseling strategies in the context of newborn screening.

P0301. Pyrosequencing™ assess the most common Cystic fibrosis mutations

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Cystic fibrosis is a lethal recessive genetic disorder in Northern European populations affecting 1 live birth in 1600-2500. In contrast to many other recessive disorders, the carriers of CF mutations (1 in 25 Caucasians) have no biochemical or physiological alterations by which they could readily be identified. As a consequence the search for genetic markers became a matter of decisive importance. Thirteen years after the discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and the characterization of over 990 mutations, CF is now to become the first disease targeted for population-wide genetic screening.

Pyrosequencing AB (Sweden) offers a PSQ™ 96 System well suited to meet the demand for research and routine testing of mutations in the CFTR gene, providing simple, rapid, cost efficient and extremely accurate detection, of all relevant mutations, including internal controls for each analyzed position. Pyrosequencing™ is a DNA sequencing technology based on real-time detection of pyrophosphate released upon nucleotide incorporation. Highly reliable CF assays have been designed and developed for all CFTR mutations with a population frequency of ≥ 0.1% including point mutations as well as insertions and deletions. The assays are greatly condensed including multiplex design of both PCR and sequencing reactions. The assays have also been optimized to even include less common mutations in the same analysis simply by extending the number of nucleotide dispensations in each pyrosequencing reaction. In addition to these two approaches, the same assays have been used for population-based determination of carrier frequencies using allele quantification.

P0302. An automated high throughput screening system for detection of 33 clinical mutations and 7 SNPs in the cystic fibrosis gene

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Cystic Fibrosis (CF) is an inherited disorder that affects children and young adults. Causative for respiratory disease and pancreatic dysfunction are mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. We have previously offered an assay (for research use only) that detects 31 mutations in the CFTR gene. This single-tube assay is based on multiplex PCR amplification and subsequent detection of the alleles by oligonucleotide ligation assay (OLA). To meet both the list of essential mutations in the recently issued ACMG guidelines for population screening of CF carriers, and the need for high throughput screening, we revised the design of the PCR/OLA-based Cystic Fibrosis assay by including all required mutations and enabled detection on the Applied Biosystems 3100 Genetic Analyzer ®.

The new assay detects the following 33 mutant and normal alleles: S549R, S549N, R553X, G551D, V520F, del I507, delF508, 3876delA, 1717-1 G→A, G542X, R560T, 3120+1G→T,R347P,R347H,I148T, W1282X,R334W,1078delT,3849+10 kbC→T, R1162X,N1303K,36 59delC,3905insT,A455E, R117H,394delTT,2184delA,2789+5G→ A, 1898+1G→A,621+1G→T,711+1G→T,G85E. Further, the assay detects these polymorphisms recommended for reflex testing: F508C,I506V,I507V and polyT in intron 8.

The instrument enables automated DNA sequencing and fragment analysis applications using an array of 16 capillaries. Up to 192 samples can be processed on two 96-well plates within 8 hours of unattended operation.The data are analyzed with Genotyper® software in an automated fashion for final review by the investigator. The assay works with purified genomic DNA and blood samples (Guthrie cards). We have tested a reference panel of 25 CF samples from Coriell laboratories and found that all samples were genotyped correctly.

P0303. Psychological impact of the introduction of a pilot for newborn screening of Cystic Fibrosis

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The Institut de Bioquimica Clinica currently analyses 74,000 newborn/ year for PKU , CH and since September 1999 CF as a pilot . The CF protocol is based in a three-tier system: a) inmunoreactive trypsine test at 2-5 days of life ,b) in testing positives a retest at 20-40 days, c) in retesting positives a DNA test for 31 mutations , sweat test and clinical examination .

The 171,693 newborns screened for CF have generated 2,500 consultations revealing anxiety or adverse emotional reactions :

-The request of the sample for the retest . In spite of the text of the letter has been carefully reviewed. Number of recalls 1,957 (1.14 %).

-The communication of a positive retest . N= 480 (0.28 %) . At this point baby still can be heterozygous or normal , in fact only a 6.6 % of them will be CF. Parents began to be aware of the severity of the disease as well as of the limitations of treatment . Benefits of genetic counselling are hardly perceived at this time.

- The finding of the mutation in only one allele. It still raises two possibilities a CF genetic compound or an heterozygous . Results of sweat test and medical exams are very important . But the screening of the whole gene takes a long time and the anxiety persist.

- The access of the parents to Internet is specially traumatic.

In conclusion all this psychosocial components will be considered in the final pilot evaluation.

P0304. DNAH3: Characterization ofthe sequence and mutation search in patients with Primary Ciliary Dyskinesia

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Primary Ciliary Dyskinesia (PCD) is an autosomal-recessive disorder with an incidence of 1/20'000, characterized by dysmotility of cilia/flagella. In addition to upper respiratory tract infections, bronchiectasis and male subfertility, 50% of cases show situs inversus (Kartagener syndrome, KS). Our linkage analysis (Blouin et al. EurJHumGenet 8:109-118) indicated extensive locus heterogeneity, in concordance with the variety of ultrastructural defects of cilia/flagella. We identified potential loci on chromosomes 3p, 5p, 8q, 11p, 15q, 16p, 17q and 19q, colocalizing with genes for dyneins, the major proteins of dynein arms defective in 50% of patients and therefore strong candidates for PCD. Our most suggestive/nearly significant linkage interval on chr.16p near marker D16S748 (NPL score =2.96 on families with dynein arm deficiency) contains the gene for axonemal dynein heavy chain DNAH3. We report here characterization of the DNAH3 gene and mutation search by sequencing all exons in patients with PCD. Genomic and transcriptional organization were determined by RT-PCR and searches of genomic sequences and ESTs. The gene spans 200 Kbp and is composed of 62 exons coding for a 4410 residues protein, highly homologous to paralogues and orthologues. Mutation search in 7 patients with PCD showing allele segregation compatible with linkage to DNAH3 revealed 9 exonic variants. Screening of the translated variants in more than 100 control population chromosomes of same ethnic origin suggests that amino-acid substitutions P1197L and A3529D observed in patients with KS and ciliary ultrastructural deficiency on either inner dynein arm/stroke or both dynein arms might be pathogenic mutations.

P0305. Primary Ciliary Dyskinesia: Mutation analysis in Dynein light chain genes mapping to chromosomes 1 (Hp28) and 22 (DNAL4)

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Primary Ciliary Dyskinesia (PCD) is an autosomal recessive disorder affecting the ultrastucture of respiratory tract cilia and spermatozoa flagella, causing dysmotility to immobility and male sterility. When respiratory infections and bronchiectasis are associated with situs inversus (half of cases), the disease is referred to as Kartagener Syndrome. PCD is genetically heterogeneous, with only a few mutations having been described to date. Our linkage analysis in 31 families (Blouin et al. 2000, EurJHumGenet 8:109-118) failed to reveal a single major locus, but suggested linkage to several regions containing candidate genes for PCD. These candidates include dynein genes, the major elements of the dynein arms, such as the novel human light chain genes Hp28 (chromosome1p35) and DNAL4 (22q12-13). For Hp28, there is suggestive evidence for linkage in the appropriate genomic interval on chr.1p (NPL=1.37). We screened 54 unrelated patients for mutations in these two genes with SSCA; electrophoretic variants were sequenced. No obvious pathogenic mutations were revealed, but nucleotide variations were observed at both loci. In Hp28 (previously screened in 7 patients: Pennarun et al., 2000, EurJHumGenet 9:P1584), two nucleotide changes were detected: A65V in exon 2 was later confirmed as a frequent polymorphism in a control population (CEPH), whereas the second variant was located in an intron (IVS3-10). In DNAL4, variants were observed in intron1 (IVS1+31) and in intron2 (IVS2+20). These studies suggest that neither Hp28 nor DNAL4 are frequently mutated in PCD.

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Authors CG and CA contributed equally.

P0306. Genotype-phenotype correlations in a group of Yugoslavian adult cystic fibrosis patients

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Cystic fibrosis (CF) is the most common serious genetic disorder among Caucasian populations, mostly diagnosed in childhood. Since the number of adult CF patients is significant, usually two groups of patients are defined: first, with late diagnosis (at the age of 16 or older) and second, patients diagnosed before their 16th birthday (early diagnosis). In a group of 164 Yugoslavian (YU) CF patients whose DNA samples were analyzed for the presence of CFTR mutations, 9,76% (16/164) were adults. Three of 19 CFTR mutations identified in YU CF patients were found in adult patients: dF508, R334W and A120T. Complete genotypes were determined in 43,75% (7/16) of adult CF patients, while 9 of them (56,25%) had at least one unidentified CF allele. Genotype dF508/dF508 was found in 31,25% (5/16) of adult patients, another 5 of them (31,25%) had dF508/non-dF508 genotype, and the group of 6 adults (37,50%) had non-dF508/non-dF508 genotype. Patients were compared considering their clinical data (sex, age at diagnosis, age, lung function tests, sweat test, pancreatic status) and determined genotypes.In this work, authors will discuss possible connection between genetic features and manifestations and prognosis of the disease in analyzed group of YU adult CF patients.

P0307. Molecular diagnosis of Cystic Fibrosis in Belarus

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Several years ago the prevalence of delta-F508 mutation has been studied in 2,598 newborns chromosomes from Belarus population. The frequency of delta-F508 mutation heterozygote carriers was 0.014 or 1:72. As this mutation accounted for 62% of CF alleles in our patients, so CF incidence had to be 1:8000. Then a whole population pilot screening of 146 701 newborns, based on initial estimation of IRT at cut-off 70 ng/ml followed by direct CF gene analysis of positive samples, confirmed the expected frequency, which is much less then 1:2500. Examination of 232 CF chromosomes from Belarus patients for the presence of the mutations, originally identified in European population, has shown that delta-F508 mutation covered 61,2% of CF chromosomes, N1303K - 2.5%, G542X - 1.3%, W1282X - 0.9%, R334W - 0.4%, R347P - 0.4%, S549N - 0.4%, R553X - 0.4%. Mutations G551D, R560T, 621+1GT, 520F, DI507, 1717-1GA, R117H, A455E, 3849+10kb were not found. Recently a large genomic deletion, spanning introns 1-3 of CFTR gene, was identified and termed CFTRdel2,3(21kb). Our data show that this mutation, frequently observed in Central and Eastern Europe, is particularly common in Belarus - 6% of all CF chromosomes. Thus the total detection rate of the found mutations is 73,7%. The first trimester prenatal diagnosis has been performed in 34 CF-families by means of direct mutation analysis and combined analysis using the intragenic polymorphism IVS6a-GATT in the CFTR gene. Eight affected fetuses and 15 carriers were detected.

P0308. Analysis of candidate genes in the region of the cystic fibrosis modifier 1 (CFM1) locus.

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Cystic Fibrosis (CF) is an autosomal recessive disease, most common among Caucasians. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. There is considerable genotype-phenotype association but clinical variation observed in CF is also determined by additional, secondary genetic modifiers and environment factors. We previously demonstrated the presence of a CF modifier locus (CFM1), contributing to the predisposition of meconium ileus (MI). Association studies (transmission disequilibrium tests, TDT) using MI and non-MI CF families with at least one affected child led to refinement of the region on human chromosome 19, region q13.2. Of 18 local markers tested, the strongest association with MI was detected for microsatellite in intron one of the KCNN4 gene. While detailed analysis of KCNN4 is in progress, we have extended our analysis of genes in its immediate proximity. A gene (NM019108) immediately distal to KCNN4 has been analyzed as candidate for CFM1. This predicted gene consists of 14 exons, spanning 23 kb. It is partially confirmed by EST data. Sequencing analysis of the exons of this gene from MI-discordant sibpairs revealed 3 non-coding sequence variants. We have performed preliminary studies one of these variants (648+46T/G) and an intragenic, complex microsatellite marker in MI and non-MI CF patients and families, respectively. No significant allelic association could be detected with these two markers but studies will continue with the remaining ones.

P0309. Rare Mutations and Polymorphisms of the Cystic Fibrosis Gene in Patients with Alcoholic and Early-Onset Idiopathic Chronic Pancreatitis

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The pathogenesis of chronic pancreatitis (CP) is poorly understood. Genetic studies identified mutations in the cationic trypsinogen gene, the serine protease inhibitor, Kazal type 1 gene, and the cystic fibrosis (CFTR) gene in patients with CP of different etiologies. The aim of the present study was to perform a comprehensive DNA testing of the CFTR gene in patients with early-onset ICP (ICP) and alcoholic CP (ACP). Furthermore, single nucleotide polymorphisms (SNP‘s) induced alterations of motif scores of the serine/arginine-rich (SR) proteins SF2/ASF, SRp40, SRp55, and SC35 were analyzed using score matrices. CF causing mutations were found in 4/14 ACP patients (29%; 6.5 times the expected frequency, $p < 0.05$) and in 5/13 ICP patients (39%; 8.7 times the expected frequency, $p < 0.05$). 2 (14%) ACP and 4 (31%) ICP patients were compound heterozygous. The frequency of SNP’s was increased in patients with ACP and ICP. The SNP nt2694T/G in exon 14a reduces a SRp40 score motif and generates a new SRp55 high score motif. The SNP nt4521 in exon 24 eliminates a SC35 motif. The intronic SNP nt3041-92G/A eliminates a SRp55 and a SC35 motif and SNP nt3601-65C/A eliminates a SRp55 motif but increases a SC35 motif score. Mutations and SNP’s of the CFTR gene are associated with ACP and ICP. The most frequently identified SNP‘s clearly change the motif scores of different SR proteins, supporting the idea that the combination of mutations and SNP’s may be important in the pathogenesis of CP by affecting splicing efficiency.

P0310. Routine analysis of the CFTR IVS(8)T polymorphism discloses two pathogenic mutations

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Cystic fibrosis (CF) is the most common autosomal recessive disorder in the Caucasian population, caused by hundreds of mutations in the cystic fibrosis conductance transmembrane regulator (CFTR) gene. Routinely, we use an Oligonucleotide ligation assay (Applied Biosystems) to screen for 31 mutations in the CFTR gene, including 24 of the most common. Additionally, CFTRdele2,3(21kb), which occurs with a frequency of 1% in our population is analyzed by multiplex PCR. With regard to CBAVD the polymorphism IVS(8)T were examined by PCR/restriction anaylsis and subsequent PAGE. Generally, the poly-T tract in intron 8 exists in three variants, 5T, 7T, and 9T. The 7T and 9T variants generate a predominantly normal transcript, whereas the 5T allele reduces the level of functional CFTR and is associated with an inherited form of infertility in males (CBAVD). The existence of further allelic variants (3T, 6T and 8T) was shortly mentioned in an abstract last year. From our CF patients two out of 125 exhibited an abnormal migration pattern in the PAGE of IVS(8)T. In one patient PAGE results suggested in addition to a 9T allele a 6T allele. Sequencing, however, disclosed the 6T allele as a 7T allele carrying a splice site deletion (IVS-1delG). In the other patient PAGE displayed a 7T and 9T allele with an abnormal heteroduplex formation. In this case a IVS8-2A→C transversion was discovered in cis with the 7T allele. We therefor conclude that any atypical T variant has to be carefully analyzed for the underlying mechanism.

P0311. Prevalance of MEFV gene mutations in FMF phenotype II patients with renal amyloidosis

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Familial Mediterranean Fever (FMF) is an inherited inflammatory disease that is principally recognized in Jewish, Armenian, Turkish and Middle Eastern Arab populations. The disease is characterised by recurrent febrile episodes of fever and serosal inflammation manifested by sterile peritonitis, pleuritis and synovitis. Amyloidosis of the AA type is the most severe manifestation of the disease. Individuals with two MEFV mutations may be divided into three clinical categories; in the group of phenotype I FMF patients; the amyloid development appears after the beginning of symptoms like, fever, abdominal pain and inflammatory attacks. In some cases, renal amyloidosis may develop before other manifestations of FMF. In this group of patients which are named as phenotype II, the disease is asymptomatic until the development of renal amyloidosis. The last category, phenotype III, includes clinically unaffected gene carriers. The aim of the present study is to confirm the presence of MEFV mutations in phenotype II patients. 25 paraffin blocks from patients with renal amyloidosis and who were deceased which are thought to be phenotype II were analyzed for MEFV gene mutations. The distribution of the four most common mutations among phenotype II patients was; 38 % for M694V, 8 % for M680I, 4 % for V726A and 4 % for E148Q. The distribution of the four common mutations among FMF phenotype I patients (M694V 51.55 %, M680I 9 %, V726A 2,88 % and E148Q 3,55 %) was not significantly different from that found in phenotype II patients.

P0312. The differential contribution of MEFV mutant alleles to the clinical profile of familial Mediterranean fever

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurring attacks of fever and serositis. Five sequence alterations (M694V, V726A, M680I, M694I and E148Q), in the MEFV gene, account for the majority of FMF chromosomes. The wide clinical variability of the disease has been related to MEFV allelic heterogeneity. M694V homozygotes have a severe form of the disease. Mutations E148Q and V726A have reduced penetrance. The clinical features, associated with the M680I and the complex V726A-E148Q allele, are not well defined. This study further characterizes the phenotypic profile associated with the major MEFV mutations. We investigated 220 FMF patients, in whom both FMF alleles have been identified, and found that different genotypes are characterized by a specific allelic related clinical profile and penetrance. Homozygotes for the M694V mutation and the complex V726A-E148Q allele are the most severely affected and often endure renal amyloidosis. Homozygotes for the M680I and V726A alleles and compound heterozygotes for either the M694V or the V726A-E148Q alleles in combination with either the E148Q, the V726A or the M680I alleles are significantly less severely affected. The morbididity associated with the complex V726A-E148Q allele by far outweighs that associated with the V726A allele, bearing evidence to the fact that the E148Q mutation is not a benign polymorphism. These findings increase our understanding of the role of allelic variability in disease expression.

P0313. Male gender increases susceptibility to amyloidosis in FMF patients homozygous for the M694V-MEFV mutation.

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder characterized by recurrent attacks of fever and serositis and predisposition to renal amyloidosis. Five sequence alterations (M694V, V726A, M680I, M694I and E148Q), in the MEFV gene, account for the majority of FMF chromosomes. Differences in the clinical expression have been partly attributed to MEFV allelic heterogeneity. The M694V/M694V genotype is associated with a severe form of disease. Otherwise, a role for additional genetic and/or environmental modifiers has been proposed. Of these, male gender was found to influence disease penetrance and susceptibility to renal amyloidosis. The aim of this study was to further investigate the contribution of sex to the phenotypic profile, in FMF. We thus performed a sex-phenotype correlation analysis on a large cohort consisting of 124 FMF patients who were all homozygous for the M694V mutation, thus precluding the weight of allelic heterogeneity. Although a preponderance of male patients was documented (73: 51; 1.4), the overall male:female ratio was significantly higher among patients with amyloidosis (32:15; 2,1) than among patients without amyloidosis (41:36; 1.1). The calculated FMF severity scores were equally high among male and female patients (9.5±3.0 and 9.7±2.8, respectively). Male gender acts as an MEFV-independent factor increasing susceptibility to renal amyloidosis (OR = 2.37; 95% CI = 1.06-5.26).

P0314. Familial Mediterranean fever - Results of MEFV gene analysis and detection of two novel mutations

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Familial Mediterranean fever (FMF) is an autosomal recessive disorder (MIM 249100) characterized by recurrent episodes of fever and serosal inflammation with peritonitis, arthritis, erythema. Amyloidosis causing renal failure is the most severe complication of the disease which primarily affects populations of Mediterranean extraction.
In 1997, a gene for FMF (*MEFV*) was identified. The *MEFV* gene product (pyrin/marenostrin) consists of 781 amino acids encoded by 10 exons. The specific protein function remains unknown but the expression in polynuclear leukocytes suggests an essential role in inflammation processes.
Several FMF studies helped to identify *MEFV* mutation hot spots. Based on these data, a stepwise procedure was applied for FMF routine diagnostics. Stage 1 consisted in sequencing of exon 10 where most *MEFV* mutations are found. Stage 2 meant was analysis of exons 2, 3 and 5, the location of several additional FMF-associated mutations. In Stage 3 the remaining exons were sequenced.
Our diagnostic strategy applied to 94 cases resulted in the identification of one *MEFV*-mutation in at least 50 cases. In 31 patients the genetic cause of the disease could be verified. In addition, two novel mutations associated with FMF were found. The mutation E148V (in exon 2) affected one patient, the mutation I591T (in exon 9) was detected in two unrelated patients.
Our three-level FMF diagnostic study proved to be efficient without missing the identification of novel mutations. The availability of the molecular test has major clinical implications considering the prognostic value of some *MEFV*-mutations and the variability of the disease phenotype.

P 5. Cytogenetics

P0315. Molecular Cytogenetic Study of Cases With Short Stature

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Among 150 cases with short stature and delayed puberty referred to the outpatient clinic of Human Genetics Department, National Research Centre, during two years period, we selected 25 Egyptian girls with phenotype far more severely affected than expected in Turner syndrome.
Initially, the karyotype in some cases was thought to be 45,X with a chromosome marker. However, re-examination with FISH probes showed 14 subjects with 45,X/46,X r(X) karyotype. Seven subjects with 46,XisoX(q) karyotype; 2 subjects with 45,X karyotype; and 2 subjects with 46,XX karyotype. Tiny ring X was present in 5 cases, and inactivation was proved by molecular cytogenetic techniques. The clinical picture of cases with ring (X) chromosome includes mental retardation in 8 patients (the non-verbal I.Q. tends to be lower

than the verbal I.Q.) and learning disability in 3 cases. Dysmorphic features are found in 3 cases and limb anomalies in 5 cases.
Our results showed that the severe phenotype was present in the cases with tiny ring (X) chromosomes suggesting mutation in the X chromosome inactivation pathway and that the inability of these rings to inactivate was responsible for the severe phenotypes. We confirm the advent of in situ hybridization with chromosome specific DNA probes in identifying small structurally abnormal chromosomes.

P0316. Pure partial trisomy 4q caused by a tandem direct duplication dup(4)(q27qter)

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We report a 3 year old boy with a de novo direct tandem duplication dup(4)(q27qter) confirmed by FISH using whole chromosome 4 painting probe. Our patient showed a particular dysmorphic phenotype, an epilepsy, a sensorioneural deafness and a moderate mental retardation. Magnetic resonance imagine of the brain showed a dilated cerebral ventricles. No other visceral malformation was detected.
Few cases of pure partial trisomy 4q was described. According to this new case and previously published data, we support that the renal malformation obseved in meny cases of 4q trisomy is related to a region proximal to 4q27, neurosensorial deafness is related to 4q31q33 region and suggest that trisomy of the 4q33q35 region is associated with minor clinical effect.

P0317. Effect of hydroxyurea and catalase on chromosomal damage and G2 arrest in Fanconi Anemia lymphoblasts from groups FA-A, FA-B, FA-C, FA-D1 and FA-E.

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Fanconi Anemia(FA) is heterogeneous, and 8 complementation groups have been discovered: FA-A,B,C,D1,D2,E,F,G. We showed that in lymphoblasts FA-A and B damaged with mitomycin C(MMC), Hydroxyurea(HU), added in G2, produces potentiation with a striking increase of chromosomal aberrations(CA). Here, we investigated whether this potentiation is due to dNTP pool depletion or free radicals (FR) produced by HU. Normal lymphoblastoid cell cultures and from FA-A,B,C,D1 and E were grown. Half cultures were treated with 10un/ml MMC for 24 hours; during G2 phase all cultures were treated with HU 2mM, and half were treated with Catalase 0.6 mg/ml which eliminates FR. Cells were processed to analyze: a) CA in 50 cells per treatment per cell line; b) Proportion of cells in G2/M by flow cytometry. Cultures were done in triplicate, data were compared by varianza and Tukey or Tamhane test and Z of proportions. Increased CA were found in all cell lines, but MMC-HU potentiation was observed only in FA-A (300%) and FA-B (100%). Catalase added to FA-A and B diminished CA frequency to 5-10%; however, CA frequency never returned to that MMC-induced. These data suggest that MMC-HU potentiation in FA-A and B is through alteration of dNTP pool. The proportion of G2 cells increased in MMC-treated cultures and decreased with HU in all FA cell lines, indicating that the restriction point of G2 is normal, and HU reduces the proportion of G2 cells, possibly because cells fail to arrive to G2. All data suggest an abnormal postreplicative repair in FA-A and B.

P0318. FISH at complicated cytogenetic cases

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Three cases of complicated chromosomal rearrangements elucidated by FISH are presented:
-Familial isodicentric supernumerary chromosome 15 and familial inverted chromosome 18 at families with reproductive failure.
-Pseudodicentric chromosome 18 at fetus with Edwards syndrome.
Role of FISH as the adjunct of classical pre- and postnatal cytogenetics in routine practice is discussed.

P0319. Blepharophimosis Is The Most Constant Clinical Feature In 14qter Microdeletion Syndrome

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Mental retardation is a distressing disorder affecting approximately 3% of the population. Among these, cytogenetic anomalies explain about 30% of patients with more severe mental retardation. Using conventional methods, detection of subtle structural aberrations is clearly limited to 6-10Mb. According to Flint et al.(Nat Genet,1995) a substantial percentage of mental retardation is caused by subtelomeric abnormalities. A couple of methods is now available to establish diagnosis of submicoscopic deletions or more complex rearrangements. We observed a young lady in which the dysmorphic phenotype in spite of a normal karyotype remained highly suggestive for a chromosomal aberration: the most impressive feature was the orbital region with blepharophimosis, epicanthal folds, puffy eyelids, a long shaped face with a pointed chin and small ears. The hands were small with tapering fingers, body length was at the 90th percentile range, no microcephaly was observed. Hypertrichosis was seen only in the first few years. Mental disability was in the mild range, with surprisingly good speech development. Finally, diagnosis was done at the age of 12 years using a commercial multitelomere FISH-kit (Cytocell). It revealed a subtelomeric de novo deletion on chromosome 14q. This result in the patient and the normal parental karyotype were confirmed by a new strategy recently described by Fauth et al. (Hum Genet,2001). Considering the results of other groups we suggest blepharophimosis to be the most constant and impressive clinical feature of this rare condition and we recommend therefore to rule out 14qter deletion in all patients with equivocal blepharophimosis syndromes.

P0320. FISH assessment of sperm aneuploidy frequencies in ICSI patients with severe oligoasthenotetraozoospermia (OAT)

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The aim of this study was to examine chromosomes in sperm by fluorescence-in-situ-hybridisation (FISH) with considerable differences in disomy frequency for the chromosomes 13, 16 and 21. On date 4 patients with oligoasthenotetraozoospermia were involved in our study. FISH procedure was made by standard protocol, using probes for chromosome 13 and 21 (locus-specific probes) and a probe for chromosome 16 (centromeric satellite probe). For each patient 2000 sperms were analysed.
In the patients, the incidence of chromosome 13 and 16 disomy ranged from 0.08% to 0.21%, and from 0.05% to 0.13% respectively. In the case of chromosome 21 we observed substantially higher rate of disomy in one of our 4 patients. In three patients, the 21 disomy ranged from 0.1% to 0.29%, while in the fourth patient, the 21 aneuploidy reached 4.1%.
Our results support the hypothesis of positive correlation between OAT and higher disomy rates. However, this correlation is not linear and absolute, among patients with severe OAT we can observe also high proportion of men with normal range of disomy. Only one of our patients had a substantially higher rate of 21 disomy than men with normal sperm quality. The other three patients had normal disomy rates of chromosomes 13, 16 and 21 even suffering from OAT. According to our results and also results from literature, we recommend a more intensive prenatal control of pregnancies, originating from the ICSI method. The study will be continued and we will include more data in the poster.

P0321. Molecular cytogenetic analysis of a constitutional de novo interstitial deletion of chromosome 12 (del(12)(p12.3p12.1)) in a boy with developmental delay and minor anomalies

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We describe the case of a 6-month-old boy with psychomotor

retardation, craniofacial dysmorphism, cleft lip and palate, as well as hearing and visual impairment. Analysis of G-banded metaphase chromosomes from the propositus revealed the presence of an interstitial deletion of the short arm of chromosome 12 (del(12)(p12.3p12.1)). To define the deletion extent on the molecular cytogenetic level, we hybridized BAC clones mapped to band 12p11, 12p12 and 12p13, respectively, to metaphase chromosomes of the proband. Our FISH results demonstrate that the deletion on chromosome 12 spans the region flanked by BACs RP11-174G6 (12p12.3) and RP11-325D10 (12p12.1). As deduced from the map position of these BAC clones in the Ensembl contigs, the deletion encompasses about 12.5 Mb. According to the Ensembl database, the deletion is flanked by markers D12S1832 and G62375.
Up to now seven patients with de novo interstitial deletions involving bands 12p12 have been described in the literature. Some of this patients had a number of clinical symptoms in common with our patient, like psychomotor retardation, microcephaly and dysmorphic facial features. Diverse cardiovascular anomalies and eye abnormalities were also observed in several cases, but all these features are equally found associated with other chromosomal aberrations. It is currently unknown, if among rare structural chromosome aberrations like these interstitial deletions within 12p breakpoint cluster regions can also be found as is the case in the more frequent category of microdeletions responsible for microdeletion syndroms like for example DiGeorge and Prader Willi syndrome.

P0322. Tandem duplication of the NF1 gene detected by high-resolution FISH in 17q11.2 region

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The gene responsible for Neurofibromatosis type 1 (NF1), which has been mapped to 17q11.2, has one of the highest observed mutation rates. We have previously shown by means of high resolution FISH that a number of the loci flanking the NF1 gene are duplicated, in line with the previous identification of NF1 REPs by other groups. We here report on a direct tandem duplication of the NF1 gene identified in 17q11.2 by means of high-resolution FISH. FISH on stretched chromosomes using locus-specific probes revealed the duplication of most NF1 gene from the promoter to 3'UTR, but with at least the lack of exon 22. Fiber FISH using PACs/BACs specific for the NF1 gene, including the 5'UTR and 3'UTR and flanking regions, visualized the direct tandem duplication of NF1 and showed the similar, but not identical genomic organization of the duplicon copies. A duplicated NF1 gene was also found at orthologous chromosomes loci in chimpanzee and gorilla, suggesting that the duplication occurred before the divergence of great apes. We hypothesize that the NF1 intrachromosomal duplication may contribute to the high whole gene mutation rate by gene conversion, an unlikely mechanism in the case of NF1 pseudogenes containing only limited portions of the NF1 gene. The functional activity of the NF1 copy remains to be investigated. Detection of the NF1 duplicon by high-resolution FISH may pave the way to filling up the gaps in the human genomic sequence of the pericentromeric 17q11.2 region.

P0323. A case of Wolf-Hirschhorn syndrome diagnosed by FISH

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The Wolf-Hirschhorn syndrome (WHS) is caused by partial deletion of chromosome 4p. In a subset of cases the deletion may be so small that it may escape detection by standard chromosome analysis. The syndrome is characterized by severe growth and psychomotor retardation, microcephaly, 'Greek helmet' facies and closure defects . We report on a 4-month-old girl whose clinical signs strongly suggested WHS. She is the product of the third pregnancy of young healthy non-consanguineous parents. The parents had had a miscarriage at 4.5 months gestation and a child who had died one month old.with cleft lip and palate and other congenital malformations.
Clinical findings at the age of four months included severe postnatal growth retardation, microcephaly, dysmorphic facies (hypertelorism, iris coloboma, cleft palate, micrognathia), heart defect , right renal agenesis and functional neurological abnormalities .
Chromosomal analysis by G-banding at 450 - 550 bands resolution indicated a normal 46,XX karyotype. Because clinical signs were very suggestive of WHS, FISH studies were performed using two DNA probes for chromosome 4p16, one BAC 19G2 and one from Vysis Inc. The patient demonstrated a typical 4p16 deletion spanning both probes.
To our knowledge, this may represent the first case of a FISH diagnosis established in North-Eastern Romania. The diagnosis was possible by collaboration with the Molecular Cytogenetic Unit at the Dresden University. On the basis of the reproductive history of the parents, it makes sense to expect a cytogenetically cryptic balanced chromosomal rearrangement in this family. Parental FISH testing is presently in working.

P0324. Ring autosomes database of National Registry of Chromosomal Abnormalities: the study of mitotic instability of constitutional ring chromosomes

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Twelve patients with ring chromosomes 4, 13, 15, 18, 21 and 22 in constitutional karyotype were registered in Belarus National Registry of Chromosomal Abnormalities among the individuals who were cytogenetically studied in Republic Genetic Center during 1983-2001 years. In theory, phenotype abnormalities of ring chromosome carriers are associated with loss of chromosomal material. However, in part of cases telomere pairing without deletion of important genetic material takes place in ring formation, and the mitotic ring instability can cause the phenotypic anomalies, independently what chromosome is involved. The phenotype of such a "general ring syndrome" consists of growth failure without malformations, few or no minor anomalies, mild-moderate mental retardation.
We studied the mitotic behavior of various size ring chromosomes in non-mosaic karyotypes
- to examine the supposition that the size of ring influences on it stability and
- to evaluate the correlation between ring size and instability of somatic cells.
Cytogenetic analysis showed dicentric rings of twice the size, smaller rings, three- and tetracentric rings, double size and single size rings simultaneously, two single size rings, poliploid (three-, tetra- and pentaploid) metaphases, metaphases with 45 chromosomes without ring and other abnormalities. Mitotic instability of ring chromosomes in vitro as well as the lability of rings in vivo may lead to high cellular death rate and interfere with normal development. Our data further support the suggestion that larger ring configurations are more unstable than smaller ring chromosomes. More essential contribution of larger rings instability to formation of «general ring syndrome» is discussed.

P0325. Alternative mechanisms in the pathogenesis of true hermaphroditism

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True hermaphroditism (TH) shows genetic heterogeneity and several genetic abnormalities have been associated with the dual gonadal development: point mutations in the SRY gene in 46, XY patients, trisomy for chromosome 22 and hidden mosaicism for a Y chromosome or Y sequences in 46, XX patients. We performed cytogenetic and molecular analyses of Y sequences in DNA from blood leukocytes and gonadal tissue in 12 Mexican TH. The karyotypes did not revealed chromosome abnormalities. Nine cases showed a 46, XX karyotype and eight of them were SRY negative in leukocytic and gonadal DNA. In case 1, also a 46, XX TH, PCR and FISH analysis performed in an ovotestis homogenate and ovarian region revealed the presence of SRY, indicating a gonadal mosaicism. Other Y sequences (PABY, ZFY, Ycen, Yqh) tested in

all 46, XX TH including patient 1, were negative in both tissues. In-patient 4, PCR revealed the presence of Ycen and Yqh and absence of Yp sequences in leukocytic, ovotestis and fibroblastic DNA. FISH analysis confirmed the presence of a Y mosaicism and each Y positive cell showed two X centromeres, demonstrating a second cell line with 47, XX del (Y) (p?). In 3 patients with a second cell line with a Y chromosome (patients 10-12), all Y sequences tested were amplified. Sequence analyses in the 4 SRY positive cases (patients 1, 10-12) was normal, excluding SRY mutations. We confirm that the presence of hidden Y mosaicism must be discarded in the molecular study of TH.

P0326. Mosaic case of Patau Syndrome with karyotype 46,XXt(13;13)/45,XXt(13;15)/46,XX

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The index patient – a 8,5-year-old girl - was the second child in the family (the first child was a healthy boy). She was born when her mother was 21 years old at 36 weeks of gestation.Her weight was 3250g, length – 57cm. Her early milestones of development were retarded.The girl sat at 10 months, walked without any aid by 18 months and said her first words at the age of 2,5 years.
At the age of 8,5 years her height was 138 cm, weight – 25 kg, cranial circumference – 52,5cm.
Dysmorphic features included: hypotelorism, enophtalmos, progenia, pro-minent sharpended nose, deformed ears. Speech and mental development were retarded. Her behavior was characterized by inappropriate smiles.
The proband's karyotype was: 46,XX t(13;13)/45,XX t(13;15)/46,XX. Her mother had a normal 46,XX karyotype. Her father's karyotype was unknown.

P0327. A natural equivalent to human satellite DNA-based artificial chromosome persists over 140 years, in a three-generation family

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Human artificial chromosomes represent an attractive tool for gene therapy. Recently, we demonstrated that human satellite DNA-based artificial chromosomes (hSATACs) could be generated via amplification-dependent de novo chromosome formation induced by integration of exogenous DNA sequences into the centromeric/rDNA regions of human acrocentric chromosomes. Human SATACs have been successfully constructed in different cell types from exogenous DNA, and "neutral" endogenous sequences of the short arm of chromosome #15. These artificially generated accessory chromosomes carry predictable DNA sequences and they contain defined genetic information. We suggested that hSATACs composed of rDNA and non-coding satellite DNA sequences that lack transcription units for undesired and unknown genes can be regarded as genetically "neutral" and hence are prototypes of safe or low risk artificial chromosome vectors.
Here, we report the existence and characterization of a natural equivalent to human satellite DNA-based artificial chromosomes. This small stable accessory chromosome derived from the centromeric/short arm region of human chr #15 is carried by healthy members of a family, and remained apparently unchanged at least in three generations. The stable persistence of this natural accessory chromosome supports the assumption that hSATACs derived from the centromeric/short arm region of chr #15 may be suitable long-term gene expression platforms without detrimental consequences.

P0328. Paternal origin of der(X)t(X;6) in a girl with trisomy 6p

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Here we report on the girl with trisomy 6p due to unbalanced t(X;6) and unusual mosaicism involving abnormalities of chromosomes 6 and 10 in the mother. Our patient is 6-year-old girl with moderate mental retardation, short stature, failure to thrive and mild facial dysmorphism. The chromosome analysis of the proband showed a 46,X,der(X)t(X;6)(q22; p11) karyotype. The derived X was late replicating in all investigated cells with variable spreading of X chromosome inactivation onto translocated 6p. The normal karyotype was observed in the father, while the mother presented 46,XX/ 46,XX, der(10)t(6;10)(p11;p11). The mother is a mosaic with unbalanced t(6;10) in 4,7% of cells. To the best of our knowledge, this unusual mosaicism has not been reported yet. We suggest that chromosome constitution in the mother is due to postzygotic recombination involving chromosome 6 and 10 at S/G2 phase of the cell cycle. In order to understand the mechanism of formation of der(X) in the proband we performed DNA polymorphism analysis. The molecular analysis revealed that chromosomes X and 6 involved in the rearrangement are of paternal origin. This work was supported by grant from Ministry of Science and Technology Republic of Croatia (project no. TP-01/072-01).

P0329. Cytogenetic, FISH and molecular analysis of the stable dicentric X chromosome

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We present the results of cytogenetic and molecular study in a 5-years old girl with mild dismorphism, grow retardation and structural rearrangement of X chromosome. Both parents presented normal karyotype. Chromosome analysis revealed one normal and one aberrant X chromosome. The rearranged X is a large submetacentric with one primary constriction and two blocks of C-staining. It is composed entirely of the X chromosome material. Two mosaic cell lines were detected by interphase FISH with X chromosome specific centromeric probe. Three signals were observed in 84.5% and one signal in 15.5% of interphase cells. Replication analysis showed the normal X always early replicating while the dicentric was late replicating in all investigated cells. Molecular analysis using polymorphic DNA markers revealed that the dicentric is of paternal origin. Based on this study the karyotype of the patient is 45,X/46,X,psu idic(X)(q22.3). We suggest that dicentric is the results of postzygotic isolocal break in both chromatids of the paternal X chromosome, subsequent rejoining of broken ends, followed by inactivation of one centromere. These results point out to the importance of combined cytogenetic, FISH and molecular study in order to elucidate the mechanisms of formation of chromosomal abnormalities. This work was supported by grant from Ministry of Science and Technology Republic of Croatia (project no. TP-01/072-01).

P0330. Terminal deletion 4q in a patient with complex chromosomal rearrangement and characteristic phenotype

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We report on a 2 year old patient, who was born at term with low birth weight, brachycephaly, low set ears, full periorbital region, curved eyebrows, strabism, depressed nasal bridge, upturned nose, high arched palate and micrognathia. He had overlapping fingers as observed in trisomy 18, but no cardiac anomalies. He had severe postnatal growth retardation and profoundly delayed psychomotor development. Routine cytogenetic analysis suggested a balanced translocation between chromosomes 6;14 and a possible deletion of the short arm of chromosome 4. FISH studies were performed showing a complex rearrangement between chromosomes 4, 6 and 14. Distal segment of chromosome 6 (breakpoint q16) was translocated to the long arm of chromosome 4 (breakpoint q26). A part of the long arm of chromosome 4, distal to the breakpoint, was translocated to the short arm of chromosome 14. Studies for the Wolf-Hirschhorn locus detected two signals 46,XY,t(4;6),t(4;14),4qter-. Since no signal was detected by FISH with 4q terminal specific probe on the derivative 14, we assume a fourth break at the distal part of the long arm of chromosome 4 leading to a terminal deletion, and suggest that the long arm segment of chromosome 4 was translocated by the terminal broken end. Thus, this complex

rearrangement resulted in a 4q terminal deletion. The phenotypical features were comparable to that of the previously described patients with ࡄq- syndrome'. Our observation contributes to the phenotypic spectrum of the rare ࡄq- syndrome'.

P0331. Monozygotic twins with a trisomy 11p due to an unbalanced translocation.

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A pregnancy, complicated by a twin transfusion syndrome (TTS), resulted in the birth of female twins by cesarean section in the 30th gestational week. Since both twins showed dysmorphic features, in one more pronounced than the other, karyotyping of blood samples was done in both. This showed a 46, XX, der(15)/46, XX karyotype in both.
Fluorescent in situ hybridisation (FISH) with a panel of probes identified the additional material on the short arm of chromosome 15 as to be derived from the short arm of chromosome 11.
Since at that time, the twins showed discrepant phenotypes which we felt could not be explained by the TTS alone, FISH with an 11p-probe was performed on a buccal smear and a urine sample of both twins. This showed three signals in part of the cells with the most dysmorphic twin having a larger proportion of abnormal cells, both in buccal and bladder cells.
Meanwhile, DNA-investigations on blood samples of the children and their parents had confirmed that the twins were monozygotic.
Clinical data and results of the chromosomal investigations will be presented in detail. The children show features of a phenotype as described by Slavonitek et al (1). Mosaicism for unbalanced structural chromosomal abnormalities in live-born infants is a rare observation and its origin in these twins will be discussed.
1. Slavonitek, A., Gaunt, L., Donnai, D. Paternally inherited duplications of 11p15.5 and Beckwith-Wiedemann syndrome. J.Med.Genet. 1997;34:819-826

P0332. Prognostic impact of cytogenetics in patients with B-CLL

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B-chronic lymphocytic leukemia (B-CLL), the most common adult leukemia in Western countries is characterized by clonal chromosomal abnormalities detected in almost 50% of studied patients. The most frequent are deletions 13q14, trisomy 12, deletions 17p13 and deletions 11q22-q23.
During the last four years we performed dual color I-FISH on bone marrow and/or blood smears of 178 patients (114 males, 64 females, mean age 64,1 years) with B-CLL.
Trisomy 12 was found in 34 of them (19%), deletion 13q14 was analysed in 162 patients and proved in 85 of them (52.5%). Deletion 17p13 was found in 32 (19.6%) patients out of 163 examined. By probe LSI MLL for 11q23 region we studied 56 patients. Although this gene is not included in pathogenesis of B-CLL, deletion was proved in 10 (17.8%) of them. All DNA probes were from VYSISTM and cut-off level 2.5% was established on controls (standard deviation not exceed 0.5%). Recent studies revealed that deletions of chromosome bands 11q22-q23 including the ATM gene locus are one of the most common chromosomal aberrations in B-CLL. We will retrospectively evaluate the incidence of the deletion in our patients with ATM/FDX probe.
Correlations of the molecular-cytogenetic findings with the immunophenotype, clinical course will be presented and prognostic impact will be discussed.
This work was supported by grant IGA MZ CR NC 6470-3/2001 and CEZ J 13/98 111100004

P0333. A case with double minute chromosome carrying chromosome's 8 centromere amplicons.

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Double minute chromosomes (dms) are extensively associated with cancer cells. There are few reports of their presence in the peripheral cells of normal individuals. We report the existence of a dm chromosome in the peripheral blood lymphocytes of a 31 years old young lady, without any type of malignancy or any history of prior exposure to mutagenic agents. The patient had a leukopenia due to an anemia of vitamin B12 deficiency. The bone marrow aspiration and tephrine biopsy was not diagnostic for an MDS (FAB classification). She is followed cytogenetically for a period of five years. Peripheral blood samples were cultured accordingly to standard techniques. Chromosome preparations were stained with GTG banding technique. The karyotype was 47,XX (dm included in 50 analyzed metaphases). The dm was studied in detail with FISH. We used a specific for 8q24 region (LSI-VYSIS), that contains c-myc oncogene, a specific for MLL gene at 11q23 (LSI-VYSIS), and a centromeric for chromosome 8 (cep8-VYSIS). The visible dm was a centromere of chromosome 8 in 100% of the metaphase spreads and in 500 counted interphase nuclei as well. To the best of our knowledge this is the first case in which on a dm c-myc oncogene or MLLgene is not amplified and it contains centromer's 8 amplicons. We believe that: (1) the patient has an unusual type of MDS, (2) the amplified genes on that extra centromere of chromosome 8 propably give a favorable advantage to her mild and stable clinical course.

P0334. A solution for bloody amnios ?

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The presence of blood in amniotic fluids is classically associated with a reduced number of colonies and an increased culture time. In a prospective 1 year study, 42 bloody amniotic fluids were treated with a commercially available selective lysing solution (Vitalyse™, BioErgonomics, Inc.). All samples were run in parallel: half of the independent culture vessels were treated with Vitalyse™ prior to culturing, half were cultured using standard operating procedure. For both techniques, we compared the number of colonies per cover slip and number of days in culture. One case failed with both sets of cultures. In the remaining cases, the data collected, although not statistically significant, indicate an increase (53%) in the average number of colonies produced (9.9 vs. 6.5) and a decrease (6%) in number of days in culture (9.7 vs. 10.4) after treatment with Vitalyse™. Additionally, no difference in metaphase quality was observed. These data argue in favor of the safety and benefits of Vitalyse™ solution. Further prospective studies to support these findings are currently underway.

P0335. Cytogenetic and FISH analyses of masked and complex Philadelphia chromosome translocations in chronic myeloid leukemia.

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Bone marrow samples from 112 patients with chronic myeloid leukemia (CML) were investigated using cytogenetic methods. Fluorescent in situ hybridization (FISH) with whole chromosome paints and Vysis BCR-ABL ES probe was used to confirm and/or complete the findings. Eight variant Philadelphia chromosome (Ph) translocations were identified. Three-way Ph translocations were found in seven patients. Chromosome 4 was involved in two cases and chromosomes 3, 11, 14, 17, and 16 in one case each; in the latter patient, a ring chromosome of the translocated 9 was found (r(9)t(9;16;22)). The eighth patient had a five-way Ph translocation t(2;9;16;22;22); it involved the fusion of the 3'ADN sequence of the ABL oncogene with the 5'DNA sequence of the BCR gene on the Ph chromosome and the insertion of the 5'end of ABL in the other chromosome 22. The BCR/ABL fusion gene was detected on the Ph chromosome in all 8 cases but 2 also presented a deletion of the 5' ABL region on the derivative chromosome 9. A masked Ph chromosome was identified among the 112 patients; it involved the

insertion of ABL into BCR on an apparently normal chromosome 22, resulting in a fusion ABL-BCR gene. In conclusion, FISH analyses allowed, not only a more accurate characterization of these complex translocations with subtle abnormalities and the identification of cryptic rearrangements, but also the recognition of the deletion of the 5' ABL region, which is now regarded by some workers to be of bad prognosis.

P0336. MLL rearrangements in balanced and unbalanced 11q aberrations in patients with hematological malignancies

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Structural rearrangements involving chromosome band 11q23 have been observed in various hematological malignancies and the great majority of chromosomal changes involve the MLL gene. More than 50 different chromosome bands have been found to be implicated in these rearrangements, and identification of MLL together with its localization can be easily done by FISH with specific DNA probes.
We investigated rearrangements of MLL gene in 14 patients with different hematological malignancies (9 myeloid, 5 lymphoid), whose bone marrow cells contained in three cases balanced and in eleven cases unbalanced 11q aberrations including translocations, deletions and insertions. FISH with dual colour locus specific probe for MLL gene (VYSIS) proved in those with balanced aberrations the rearrangement of MLL gene in one case, in two cases MLL gene was translocated on the partner chromosome without rearrangement. In the cohort of patients with unbalanced 11q translocations the deletion of MLL gene was proved in three cases, rearrangement of MLL gene in three cases, in another three cases MLL gene was translocated on the partner chromosome without any change detectable by FISH and in two cases MLL gene remained on band 11q23. Whole chromosome painting probes (CAMBIO) and multicolour FISH (mFISH - MetaSystems) were used for identification of chromosomes involved in translocations.
Correlation of clinical characteristics, outcome and survival of patients according to diagnoses and various types of 11q rearrangements will be discussed.
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P0337. Clinical implications of del(20q) in patients with hematologic myeloid disorders

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Deletion of the long arm of chromosome 20 represents the most common chromosomal abnormality associated with the myeloproliferative syndrome (MPS). This aberration can be also found in bone marrow cells of the patients with other myeloid malignancies including myelodysplastic syndromes (MDS) and acute myeloid leukemia. Deleted part of 20q is rather small and its extent and breakpoints on the long arms of chromosome 20 can be identified by fluorescence in situ hybridization (FISH) with specific DNA probes.
We report findings in bone marrow cells of 34 patients (19 males, 15 females, mean age 65 years, range 21-89 years) with malignant myeloid diseases with deletion 20q determined by classical cytogenetic techniques. All cases were studied by FISH using locus specific probes for band 20q12 (LSI D20S108, VYSIS). According to the results of both cytogenetic analyses this cohort of patients was divided into two groups: in the first one the patients with deletion 20q as a single aberration (20 patients) were presented, in the second group were the cases with deletion 20q and other chromosomal changes (14 patients). We correlated hematologic findings with the results of cytogenetic examinations and time of survival in both groups of the patients. The prognostic value of deletion del(20q) in our cohort in comparison to those published in literature will be presented. Deletion del(20q) as a single aberration is a sign of better prognosis for the patients with MPS and/or MDS.
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P0338. Importance of genetic investigation in couples involved in "in vitro fertilisation"(IVF)

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PROBLEM: The aim of study was to evaluate the contribution of chromosomal abnormalities and gene mutations (AZF and CFTR) in cases of decreased fertility.
RESULTS: The frequency of chromosomal aberrations in examined group was 6,5 % (29/444). We detected 7 (1,5 %) cases of balanced chromosomal rearrangements, 2 (0,45 %) cases of cytogenetic deletion of Y chromosome, 1 (0,23 %) case of inversion, 1 (0,23 %) case of marker chromosome, 13 (3,0 %) cases of gonosomal mosaicism and 5 (1,13 %) of gonosomal aneuploidies. The prevalence of AZF deletion at azoospermic men in this group was 3,91 % (5/128) and CFTR gene mutation was 4,79 % (7/146).
In a small group of pregnant patients after IVF included in our study the frequency of chromosomal abnormalities was 18,6 %.
CONCLUSION: High number of infertile couples is affected by chromosomal aberrations. It is suggested that chromosomal analyses should be performed before IVF treatment. Incidence of AZF a CFTR gene mutations was also frequent in studied group and for that reason the screening of both AZF deletion and CFTR gene mutation is recommended in azoospermic men.

P0339. Prenatally diagnosed chromosome abnormalities and ultrasound markers: A report of 537 cases

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Fetuses with chromosomal abnormalities are usually characterized by specific, minor or major, multiple anomalies accepted as sonographic markers of chromosomopathies.
Fetal karyotyping was performed for 537 patients because of fetal abnormalities on ultrasonography. The fetal karyotype was abnormal in 8.94% of cases (48/537). There were: autosomal aneuploidy in 26 cases (4.84%), structural rearrangements in 9 cases(1.67%), sex aneuploidy in 8 cases (1.49%), triploidy in 3 cases (0.56%) and marker chromosomes in 2 cases(0.37%).Unbalanced structural anomalies were frequently associated with intrauterine growth retardation (4.13%). Within the group of single sonographic anomaly, the most frequent chromosomal disorders were trisomies 21 and 18 (3.17%). Among fetuses with multiple sonographic anomalies, Turner's syndrome was detected in 3.06%, trisomy 21 or trisomy 18 in 2.04%. Chromosomal abnormalities were detected in malformations affecting:
-nervous system : 5.67%
-gastrointestinal system : 7.14%
-renal system :10.4%
-chest : 13.72%
-neck : 16.67%
-abdomen : 30.77%
-skeleton : 8.19%
-hydrops : 8.33%
Minor fetal anomalies also serve as ultrasound markers of fetal aneuploidy, the most specific is nucal translucency. It was associated with chromosomal anomalies in 13.3% of cases.

P0340. DNA analysis of Y - chromosomal sequences from different tissues in Turner syndrome patients.

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Introduction

Incidence of Turner syndrome (TS) is about 1: 2500 of liveborn girls in the Czech Republic. Chromosome Y sequences having the physiological function in males can promote the development of gonadoblastoma in undifferentiated gonads of TS patients. The risk of tumourgenesis is about 30%.
Methods
Samples of 124 Czech TS patients were examined by polymerase chain reaction (PCR) and quantitative fluorescent PCR (QF PCR) in 4 loci. Y - positive cases were furthermore tested using fluorescent in situ hybridisation (FISH).
Results
Tests of peripheral blood by:
PCR in loci DYZ3 4,8%, AMGY 3,2%, SRY 3,9%, PABY 4% and QF PCR in loci DYZ3 13,7%, AMGY 5,6%.
Detection of Y sequences from paraffin - embedded gonadoblastoma tissue in DYZ3 and TSPY loci from 2 samples. One sample was positive in both loci.
FISH with Y centromeric probe was performed on following sections: two samples from gonadoblastoma tissues and one sample from undifferentiated gonad. One sample was a mosaic 1 in 20. The others showed only sporadic positive signal.
Conclusion
The majority of hidden mosaicism is not detectable by conventional cytogenetic methods. The classical PCR combined with the detection of products on agarose gel (ethidium bromide stained) was quite unsuitable for mosaic determination. QF PCR is the most sensitive and the most precise method for the assessment of Y chromosome mosaicism in patients with Turner syndrome. It enables the most effective selection of persons under the risk of gonadoblastoma development.

P0341. Distal trisomy 14q and aorto-pulmonary window : a case report

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We report on a newborn female with partial trisomy 14q. She was born at term after an uneventful pregnancy as the first child of an unrelated couple. Her birth weigth was 2830g, length 47cm, head circumference 30cm. At birth she was noted to have an unusual face, a 3/6 systolic murmur and was admitted to the pediatric department. She had a large anterior fontanel, hypertelorism, broad nasal bridge, high arched palate, micrognathism, low set ears, short neck, overriding fingers, rocker-bottom feet. She also presented hypotonia, opisthotonos, left side earing impairment, corneal epitheliopathy due to her lagophthalmia. An echocardiogram performed on the 3rd day of life showed a large aorto-pulmonary window with significant left to right shunt which required surgical closure at one month. At two months, signs of virilisation were noticed. Endocrinologic and radiologic investigation concluded to side effects of medication. Cytogenetic analysis was done. Initial investigation demonstrated additional material on chromosome 3. Fluorescent in situ hybridization showed that this material originated from chromosome 14. Her caryotype was: 46,XX,der(3)t(p25;q24). Studies of her parents' chromosomes revealed a reciprocal paternal balanced translocation between chromosomes 3 and 14. His caryotype was: 46,XY,t(3;14)(p25;q24).
We compare her clinical features with the 8 other trisomy 14q24→qter published.

P0342. Characterization of two small supernumerary marker chromosomes (SMC) by acro/cenM-FISH - first case with partial hexasomy 15pter→15q13

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Cytogenetic analysis performed in a three year old girl resulted in a karyotype 48,XX,+2mar[25/25]. She presented the following clinical features: severe mental retardation, microcephaly, postaxial hexadactyly plus a pachygyry. Such small SMCs often are uneasy to characterize in standard cytogenetic or molecular cytogenetic approaches. Recently, we developed a probe set, using all human centromeric probes labeled in different colors, allowing the simultaneous characterization and identification of all chromosomes by their centromeric region (Nietzel et al., 2001, Hum Genet, 108, 199-204). The technique, called cenM-FISH has been extended by the introduction of an additional probe specific for the short arm of all human acrocentric chromosomes called midi54 (described in Mrasek et al., 2001, Cytogenet Cell Genet, 93, 242-248). This acro/cenM-FISH probe set revealed in the present case, that the both SMC were identical derivatives of chromosomes 15 with two specific signals for the centromere 15 specific and the midi54 probe. Additional FISH experiments using the high resolution multicolor banding (MCB) technique and probes specific for the Prader-Willi/Angelman syndrome region (SNRPN and D15S10), respectively, characterized the derivative chromosomes as dicentric iso-chromosomes i(15)(pter→q13::q13→pter). To the best of our knowledge, this is the first case with partial hexasomy 15pter→15q13. Studies to clarify the origin of the derivatives (UPD-analysis) are in progress. In summary, acro/cenM-FISH is a very useful approach for the one step identification of all human chromosomes by their centromeres and acrocentric p-arms. Supported by Herbert Quandt Stiftung der VARTA AG, Wilhelm Sander-Stiftung (99.105.1) and EU (ICA2-CT-2000-10012 and QLRT-1999-31590).

P0343. Cytogenetic studies in lymphocyte cultures from 3 members of a family affected with Werner's Syndrome

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Werner's Syndrome (WS), a rare autosomal disorder arising as a consequence of mutations in a gene coding for a protein member of Rec Q family of DNA helicases (WRN), is characterized by premature aging exhibiting chromosome instability and predisposition to cancer. In cell lines derived from WS patients both stable and unstable chromosome aberrations had already been detected, and recently it has been shown that WS cells have a slower rate of repair associated with DNA damage induced in S-phase. In the present work we performed PHA-stimulated lymphocyte cultures, with and without induction with diepoxybutane (DEB), from two brothers with WS, another healthy sister and 6 healthy controls. The purpose was to analyse the frequency and pattern of chromosome instability in patients from the same family. Our results showed a higher frequency of random chromosome aberrations in all members of the family, compared with controls; however, no consistent pattern of chromosome-specific aneuploidies and structural aberrations was detected among the 3 members of the family, supporting the hypothesis that chromosome instability is related with the disease but the selection of the chromosome aberrations involved is not genetically determined. This study also revealed that the repair associated with DNA damage induced by DEB is not significantly affected in the 3 members of the family.

P0344. Supernumerary marker 22 chromosome: Clinical, cytogenetic and molecular analysis of five patients.

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Extra structurally abnormal microchromosomes are a heterogeneous group of chromosomes also known as markers or small supernumerary chromosomes . In most cases, the extra chromosome is derivated from an acrocentric chromosome.Thus, patients have duplications, or in some cases triplications of the chromosomal segments included in the extra.
Patients, material and methods : We have studied five patients with an extra marker 22 chromosome. All patients were evaluated with GTL, CBG, NOR-Ag banding, fluorescence in situ hybridation (FISH) using several probes and microsatellite anlaysis with four different markers.
Results: Four patients had typical clinical signs and symptoms associated to the Cat eye syndrome (CES), showed peculiar face, abnormal ears, anal stenosis/atresia and some degree of mental retardation, ranging from mild to moderate. Iris coloboma, a clinical finding usually observed in CES, have not been found in none of our patients.One of them had a complex congenital heart defect and died at age of three months.Cytogenetic studies showed that one child had mosaicism for the marker chromosome, and the remaining four

children had 47 chromosomes in all evaluated metaphases. Parents were all cytogenetically normal.
There was heterogeneity on the FISH and microsatellite results demonstrating that the duplicated segment varied in size in each patient.
Comments: Our results in five patients with extra satellited 22 marker chromosome showed that the phenotype in these patients is characteristic of CES and that iris coloboma may be not present in a percentage of patients.

P0345. Chromosome 9p- deletion syndrome: 3 “de novo” cases

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The 9p- deletion syndrome was characterized for the first time in 1973. The main clinical features of this syndrome are dysmorphic facial features (trigonocephaly, midface hypoplasia, upward-slanting palpebral fissures and a long philtrum) and mental retardation.
The authors present the clinical description and the cytogenetic findings in three patients, aged 1, 4 and 43 years, with “de novo” deletions in the short arm of chromosome 9 (in 2 cases, 9p22 and 9p23 in the other). Apart from the mental retardation, the reasons for referral were, respectively, Pierre-Robin syndrome with low weight and height, severe facial dysmorphic features and apparent Down syndrome.
It is important to note that, although the symptoms are typical and diagnosis may be suspected at birth, most paediatricians are not aware of this syndrome; therefore, no provisional diagnosis or detailed clinical description are given to the cytogenetics laboratory in the majority of cases. The clinical details, together with high resolution banding patterns (especially if the breakpoint region is very close to the 9p telomere) are the best combination to avoid the underestimation of this syndrome.

P0346. A new probe set for the characterization of centromere-near rearrangements

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A variety of FISH approaches have been developed in the last decade, covering the entire human genome in multiple ways. Nonetheless, there is still a chromosomal region which is not well investigated with the presently available probe sets: the pericentric region. This region is of special interest when small supernumerary marker chromosomes (sSMC) shall be characterized. Neither whole nor partial chromosome painting (wcp and pcp) probes are informative if centromere-near euchromatic material is present on a sSMC. This question can be answered best when hybridizing centromere-near probes like BACs. At present we are working on 24 probe sets, each for one human chromosome, which consists of a centromere specific satellite probe, one centromere-near BAC in q and p (excluding the acrocentric chromosomes) and arm-specific pcp probes. These probes are used after characterizing the sSMC by cenM-FISH (Nietzel etal., 2001, HumGenet, 108, 199-204).
Up to now two cases with cat eye syndrome (CES) chromosomes [inv dup(22)(q11.2)] and three sSMC derived from #2, #16 and #22, respectively, have been characterized by centromere-near BAC probes. As expected, centromere-near euchromatic material was present in the CES-chromosomes. For the derivative #2 q-arm specific centromere-near material could be detected, while the derivatives #16 and #22 consisted only of centromere and heterochromatin. Additionally, a clinical case with a normal karyotype apart from the very small pericentric inversion could be detected using the chromosome 2 specific peri-centromere probe set. In summary, we present a probe set useful to characterize any kind of centromere-near rearrangement. Supported by EU (QLRT-1999-31590)

P0347. Role of chromosome abnormalities in recurrent In-Vitro Fertilization implantation failure

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The association between recurrent miscarriges and parental chromosome anbormalities has been well documented. Much less is known concerning the role of parental chromosome abnormalities in recurrent in-vitro fertilization (IVF) implantation failures.
The aim of this study was to evaluate the contribution of chromosome abnormalities as potential cause of IVF implantation failures.
Two hundred and forty four consecutive patients (122 couples) who had at least 3 attempts of embryos transferred without achieving clinical pregnancy, were evaluated for chromosome abnormalities. Five hundred and twenty six consecutive patients (263 couples) who had experienced 3 or more miscarriges, and 2032 consecutive antenatal chromosome diagnoses referred because of advanced maternal age or maternal anxiety (unbalanced karyotypes associated with mental retardation were excluded), served as control groups.
Chromosome abnormalities were detected in 5/244 patients with IVF failures (4.1% of the couples), in 10/526 patients with recurrent miscarriges (3.8% of the couples), and in 5/2032 (0.25%) antenatal diagnoses.
The chromosome abnormalities detected in the IVF failures group included 2 reciprocal translocations, one case of 46,XYY, one case of 46,X,del(X)(p22), and one case of 46,XY(96%)/45,X(4%) mosaicism.
The prevalence of chromosome abnormalities among the IVF patients was significantly higher than in the antenatal group ($p<0.001$), and it was similar to the prevalence of chromosome abnormalities detected in the recurrent miscarriges group.
The results of the study suggest that chromosome analysis should be considered as part of the routine investigation of patients with recurrent IVF implantation failures.

P0348. Unusual inverted duplication of chromosome 15 with terminal deletion.

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We report here the case of a female premature newborn with multiple severe congenital defects. Ultrasonographic examination at 26 weeks of gestation evidenced growth retardation, cardiac and kidney abnormalities, arthrogryposis and foot deformity. These anomalies were all confirmed at birth. Moreover, facial dysmorphism, left-hand deformity, dislocation of the hip and hypoplastic corpus callosum were found. Cytogenetic investigation performed by high resolution G banding on amniocytes, fetal blood and skin fibroblasts indicated a possible duplication of 15q. FISH analysis was performed with a painting probe for chromosome 15, with a telomeric probe and with specific 15q probes, to identify and delimit the duplicated region. An inverted duplication of the q14-26.2 region was assessed. The deletion of q26.3 was also demonstrated. The resulting karyotype was: 46, XX, inv dup del(15)(pter→q26.2::q26.2→q14). The parents' karyotypes were normal. The phenotypic alterations of the proband are only in part superimposable to those described in the literature for analogous cases. This could be due to the extent of the duplication and/or to the monosomy of a portion of q26. Chromosomal aberrations involving the human chromosome 15 (most often the q11-13 region) have been frequently reported in the literature. It has been suggested that duplicons with opposite orientation may predispose to inverted duplications and that distal deletion and a single copy region between the duplicated regions are then present. We are currently investigating the hypothesis that the rearrangement described here might be related to the recently discovered duplicons in the 15q26 region.

P0349. Asynchronous replication of bi-allelically expressed loci: A new phenomenon in turner syndrome

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Purpose: Transcriptional activity of genes is related to their replication timing; alleles showing the common biallelic mode of expression replicate synchronously, whereas those with a monoallelic mode of expression replicate asynchronously. Here we determined the level of synchronization in replication timing of alleles in subjects

with Turner syndrome. **Methods:** We used FISH for 3 loci not linked to X chromosome, in lymphocytes derived from 12 controls, 3 individuals with Turner and 4 with mosaic Turner syndrome. **Results:** In cells derived from controls, each pair of alleles replicated synchronously; yet these same alleles replicated asynchronously in cells monosomic for X chromosome derived from Turner and mosaic Turner patients. When the level of 45,X0 was low in the mosaic samples, the replication pattern of the 46,XX cells was normal. However, in samples with a high level of mosaicism, a significantly increased asynchronous replication was detected in the 46,XX cells. **Conclusion:** An altered temporal replication control in Turner syndrome may suggest an epigenetic mechanism involved in this condition affecting the aneuploid and euploid cells.

P0350. Cytogenetic analysis of 179 infertile Iranian women.

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Turner syndrome in adult is typically presented by primary amenorreha,infantile genitlia and failure of secondary sexual development. Gonadal dysgenesis resulting in primary infertility is one of the most common features of turner syndrome. Nevertheless, menstruation and pregnancy have been recorded in a few 45,X subjects. But whether on not the fertility is associated with a 46,XX cell line the germ cells is not known. We have investigated 218 female patients which were referred to our lab for primary amenorrhea or secondary amenorrhea or for R/O Turner syndrome, 35 cases showed an abnormal cytogenetic analysis. The most common chromosol abnormality was 45,X(18/35). The other karyotypes consist of: pseu/idic(X)(q22); del(15)(q11);ad d(X)(q28);r(X);iso(X)(q10);4 cases of mosaicism (45,X/46,XX/47,XXX);46X,iso(X)(q10)/45X;45X/46x;del(X)(q22.2); 2 cases of inv(9)(p11,q13). The latter cases will present a normal variant in human.

P0351. A New Case of Mosaic Supernumerary Ring Chromosome 8 Syndrome

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We described a *de novo* mos r(8) by conventional cytogenetic and FISH tecniques. The patient was referred because of neuromotor growth retardation and dysmorphic facial findings. He was a 15 year-old male, with a height of 157 cm (10th centile), and weight of 37.5 kg (<3rd centile), head circumference 55 cm (75th centile). His born weight was 1750 gr (<3rd centile), and length was 50 cm (50th centile). He had long and expressionless face with a prominent maxilla, everted lips, streched lingual frenulum which had been cut afterwards. He had big low-set ears, epicanthus, prominent root of nose, plagiocephaly, pterigium coli, camptodactyly, deep plantar creasis, overlapping of toes, hammer big toes and cryptorchidism. He had elongated thin trunk with narrow shoulders. There were cafe-au-lait on dorsal region and. Radiographic findings showed a mild kyphoscoliosis on dorsal vertebras, spina bifida on L4-L5. Magnetic rezonans Imaging (MRI) study revealed hypolasia of splenium region of corpus callosum and cavum septum pellicidum abnormalities. His IQ level was 67, he had poor speech and language development, poor awareness of necessary life skills, and had autistic-like behaviours with shyness and stubbornnness. We performed cytogenetic analysis on peripheral blood cultures by GTL banding and found 47,XY,+r[29]/46,XY[33]. We applied FISH and obtained signals on the ring chromosome with 8 painting and 8 alpha satellite probes. Oncor (Gaithersburg) digoxigenin-labelled Coatosome probes and Vysis were used for FISH analysis. The resulting karyotype after FISH analysis was mos 47,XY,+r. ish r(8)(wcp8+;D8Z1+).

P0352. Pure partial trisomy for long arm of chromosome 9

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A case of a 4-year-old boy with trisomy of the long arm of the chromosome 9 is described (46,XY, der(9), t(9;9) (q32:q12)).
The trisomy is probably the result of a translocation of the long arm of the chromosome from one homologue to the other in a prenatal gonad.
The clinical features of the child which severe developmental retardation, bird-like facies, tapered fingers, and flexion contracture of the legs are similar to those of the few cases described of trisomy of the whole chromosome.

P0353. Paracentric inversion of chromosome 1 - inv(1)(p31.2p35.2) - in a large family from northern Finland

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We describe a large family in which a paracentric chromosomal inversion was first detected in the early 80´s in a 1-year-old boy with severe mental retardation, infantile spasms and multiple congenital anomalies. He had inherited the inversion in an unbalanced form from the mother, who was carrier of inv(1)(p31.2p35.2) and who had had several spontaneous abortions. Subsequently this inversion has been independantly ascertained in six individuals of whom four were studied for developmental delay and dysmorphic features. In them no light microscopic differences could be seen at prometaphase level, when compared with the inversions of their healthy parents. This prompted us to search for the origins of the carriers of the inversion; with the help of church records a founder couple born in 1760 and 1763 could be detected, whose descendants all the inv(1) carriers are. - The cytogenetic, clinical and genealogical data of the family will be presented and the role of the inversion in the family and the population will be discussed.

P0354. Diagnosis of low rate mosaic trisomy 21 by FISH in a girl with inherited balanced translocation 6;15

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FISH can help in the diagnosis of mosaic chromosomal abnormalities. Here we present a girl diagnosed with low rate mosaicism for trisomy 21 utilizing this method.
Case report: A 12-year girl presented due to schooling problems. She was a student in the 6th grade of elementary school, very good in literature and writing, however she had poor grades in math and logical sciences. She had several pneumonias and additional lobe in right lung has been diagnosed. Otherwise, she grew normally, and the puberty occurred at an age of 11 years. Physically she had some of the features of Down syndrome, e.g. flat nasal bridge, epicantus, low set years, brachicephalia. However, she had a very well developed speech, with significant eloquence, and no suspicion of Down syndrome occurred previously.
Karyotype detected balanced translocation 6p; 7q, inherited from her father. No other chromosomal abnormality was found by karyotyping. FISH was performed on the chromosome spreads from blood lymphocytes. Out of several thousands of cells, trisomy 21 appeared in three.
Low rate mosaicism in our patient confirms that the proportion of trisomic cells influences some of the features of Down syndrome including the speech development. FISH can help to detect small number of trisomic cells in cases with unusual presentations of the syndrome.

P0355. Partial monosomy 22q11 associated with velo-cardio-cutaneus syndrome due to a de novo translocation (15; 22) (p11; q11)

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Reciprocal translocations are said to be a common finding in the cytogenetic laboratories. In most of the cases there isn't a loss of material when a breakage between two chromosomes occurs. A balanced career doesn't have any pathological signs. Sometimes during these breaks a certain amount of genetic material has been lost, or the break occurs in a specific region for some syndrome. Then the career expresses more or less dysmorphic signs, mental retardation, or organic lesions.
A 2 year old boy approached to the clinic because of motor delay and dysmorphic features. It was a second child of young and unrelated parents. The pregnancy was normal; the baby was a full-term neonate, with birth weight and length under 3rd percentile. The boy had mild brachycephaly, flat facies, hypertelorizm, up-slanted palpebral fissures, squared nasal root, big ears, micrognatia, and swallowing difficulties due to velopharyngeal incompetence. Also the boy presented heart defect, crypyorchidism, inguinal hernia, slender hands with clinodactily of fifth finger. Neurological findings revealed hypotonia, motor and mental delay. Chromosomal finding represents translocation between chromosomes 15 and 22, 46, XY, t (15; 22) (p11; q11) with a breakpoint in a VCFS site. Analysis of the mother's chromosomes showed huge satellites on chromosomes 15 and 22, which may be the cause of the breakage and translocation of chromosomes in the child. When reciprocal translocation occurs in a specific region, it produces deletions of specific genes that lead to a certain phenotype of a well known syndrome.

P0356. Pseudohermaphroditism, etiopathogenesis and classification

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Advance in experimental endocrinology, biochemistry, genetics, and molecular biology have all contributed to our understanding of the process of human sex differentiation in the last 5 year. Based on the recognition of the underlying anomaly in the process of sexual differentiation intersex disorders may be devided into abnormal gonadal determination and abnormal genital differentiation [including male and female pseudohermaphrodism(MPH & FPH)]. Abnormal gonadal determination is mainly dependent on sex chromosomal defects that can be detected by cytogenetic analysis or by the DNA probes for genes located on the Y chromosome. Individuals with ambiguous genitalia but two differentiated testis are called MPH. Females with ambiguous external genitalia but normal ovaries and normal internal genitalia are called FPH. The XX males may be divided into 3 subgroups:1) 46,XX males with SRY gene, 2)46,XX males without the SRY gene and 3)46,XX/46,XY mosaics. The insidence of 1 in 20.000 to 30.000 males with a 46,XX karyotype have been reported. We have investigated 586 infertile patients who was referred to our labfrom 1997 until 2002, we have found 39 cases (39/586)with sex reversal which was more common in the male group as male pseudohermaphrodism(24/39). The main reason of referal in the MPH group was azoospermia & in the FPH group consists of primary amenorrhea. Cytogenetic analysis is the main method for diagnosis of sex reversal as a cause of infertility, but for determining the etiology of this pattern we need molecular technology to study the SRY gene.

P0357. Evaluation of 355 azoospermics Iranian patients by cytogeneic method.

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spermatogenesis in human is a complex, dynamic process which the duration takes 70 days. The onset of release of spermatozoa occurs at about 13.5 years of age and continuing through out life into the eighth and ninth decades. In quantitative terms the out put of spermatozoa in an average adult man is 1500 to 2500 million sperm commonly present in a single ejaculate. Total absence of sperm from the semen is called azoospermia. Sperm concentration of less than 20 million/ml are classified as oligospermic. We have investigated 417 azoospermic patients in our center and a constitutional chromosomal aberration were diagnosed in 110/417(26.4%). Whereas the 47,XXY chromosome complement was the commonest (71/110),the following abnormal karyotypes were also found:46,XX; del(Y)(q11);48,XXYY; inv(Y)(pqq.2,q11.2); t(2,12); t(12,22); t(13q,14q); 45,X/46,XY; 47,XXY/46,XY and 6 patients had inv(9)(p11q13) but the pattern have been observed as a normal variant in human. We have found 4 patients with complex structural and aneuploidy abnormalities (46,X,idic(Y)(q11.31)/45,X; 47,XXY/48XXY+mar/48XXXY; 47,XXY, inv(9)(p11q13); 46,X,del(Y)(q11.21)/45,X). Pooled data from the literature showed that the frequency of chromosomal abnormalities is higher in azoospermic than in infertile. We have observed 26.4% chromosomal abnormality in azoospermia and 20% in infertile men, that is compatible with the data from literature.

P0358. Chromosome deletions in 13q: a review of 11 new cases

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Partial deletions of the long arm of chromosome 13, del(13q), are uncommon chromosome abnormalities in which a portion of the long arm is missing. The range and severity of the symptoms may vary greatly, depending upon the size and location of the absent segment. The authors present 11 cases of "de novo" 13q deletions: del (13)(q31) - 1case; del (13)(q32) - 4 cases; del (13)(q22.3) - 1 case; r(13) - 4 cases; mosaic r(13) - 1 case. All these patients were referred for cytogenetic studies due to multiple congenital abnormalities, ranging from mild to severe.
A correlation phenotype/karyotype will be established and compared with the reported literature.

P0359. Identification of supernumerary marker chromosome 20 in 3 patients.

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Marker chromosomes (MCs) are supernumerary structurally abnormal chromosomes in which no part can be identified by cytogenetic banding techniques (ISNC 1995). They are detected with a frequency of 1:1,660-1:1,040 at amniocentesis, 1:5,555 in liveborn individuals and 1:330 in mentally retarded patients. MCs are classified as de novo or familial, satellited or non-satellited and mosaic or non-mosaic. In some families, MCs are transmitted through several generations, apparently with no associated abnormalities, whereas other MCs carriers may present serious clinical symptoms, such as mental retardation, dysmorphic features and malformations. Several syndromes associated with MCs are known. Mosaic i(12p) causes the Pallister-Killian syndrome; an inv dup(22) is found in the "cat eye syndrome" and an i(18p) syndrome has also been described. MCs can now be identified by molecular cytogenetic methods but limited data currently do not permit consistent phenotype-genotype correlation to be made. In addition, variation in the size and parental origin of the marker can influence the outcome.
The occurence of a supernumerary marker chromosome 20 is rare and no common phenotype has been established. So far, there are 11 cases reported with an extra marker (20) and their phenotype varies from normal to abnormal.
We describe 3 further patients with MCs derived from chromosome 20 and identified by FISH. These MCs have been sized with BACs probes and clinical and cytogenetic findings are compared with results of case reports published to date.
This study was supported by Telethon Italia (grant E.0827)

P0360. Scanning probe microscopy studies on human metaphase chromosomes

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A better knowledge of biochemical and structural properties of

human chromosomes is important for cytogenetic investigations and diagnostics. Since conventional fluorescence microscopy has reached its limit due to the restricted optical resolution (0,2µm), we are currently working on the indroduction of different scanning probe microscopy techniques in this field: Atomic Force Microscopy (AFM) and Scanning Near-field Optical Microscopy (SNOM).
The topography of GTG-banded human metaphase chromosomes was evaluated by AFM and compared to the standard banding pattern according to the international system for human cytogenetic nomenclature (ISCN 1995), which is based on the optical properties of the chromosomes. A better resolution of the chromosomal surface in general and of the bands themselves was acchieved by applying AFM.
Another approach to make the chromosomes more accessible to DNA-probes during fluorescence in situ hybridization (FISH) and to improve the visualization of their structure consists of various biochemical treatments. We were using the protein cleaving enzyme trypsin and the polyanion heparin additionally to well established procedures like for example HCl-incubation to remove the DNA-binding proteins, histones as well as non-histones. The structural effects of these treatments were visualized by AFM and the binding structure of target- and probe-DNA after FISH was investigated by SNOM.

P0361. The cytogenetic analysis in female patients with amenorrhea and sexualization troubles

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The aim of our study is to analyse the relationship between clinical diagnosis and chromosome studies in female with amenorrhea or sexualization troubles. We divided the lot of 197 patients in 11 groups, on clinical basis. In Turner syndrome cases we found: 45,X (41) 45,X/46,XX (23) 46,XX (23) 46,XX/47,XXX (2) 45,X/46,X,r(X) (1) 45,X/46,XY (1) 45,X/46,XX/46,XY (1) 46,XY (1). Karyotypes for primary amenorrhea cases resulted in: 45,X/46,XX(5) 46,XX(25) 46,XX/47,XXX(1) 45,X/46,X,i(Xq)(1) 46,XX/47,XXX(1) 46,XY(5). In secondary amenorrhea cases we found: 46,XX (9) 45,X/47,XXX(1). Karyotype founded in Morris syndrome (4) and delayed puberty (1) phenotypes: 46,XY. In Rokytansky syndrome cases (13) we found 46,XX karyotype. In short stature cases we found: 46,XX (1) 45,X/47,XXX(1) 45,X/46,XX/47,XXX (1). The clinical cases with triplo X (2) was confirmed: 46,XX/47,XXX (1) 45,X/46,XX/47,XXX (1). In cases with Noonan syndrome we found: 46,XX (3), 45,X (1), 45,X/46,XX (1). In ovarian dysgenesia cases we found: 45,X (2) 45,X/46,XX (2) 46,XX (2) 47,XXX (1) 46,XY (1) 46,XX/47,XXY (1). In cases with clinical suspicion of female pseudohermaphroditism we found: 46,XX (10) 45,X/46,XX (1) 45,X/46,XY (1) 46,XY (1). In intersexuality cases we found: 46,XX (8) 46,XY (3) 49,XXXXY (2) 45,X (1) 45,X/46,XX (1) 45,X/46,XY(1) 46,XX/46,XY (1). Our studies results confirm the concordance between clinical aspects and cytogenetics results in Turner, Rokytansky and Morris syndromes, but difficulties in clinical diagnosis exist in females with isolated amenorrhea or ovarian dysgenesia. Instead, in intersexuality and pseudohermaphroditisms we found a discordance. In conclusion, improving of clinical examination and cytogenetic confirmation are needed in patients with sexualization troubles.

P0362. Results of chromosome studies in 664 Iranian couples with the history of recurrent early pregnancy loss.

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Approximately 15 to 20 per cent of recognized pregnancies end in a first-trimester spontaneous abortion.An estimated 0.4 to 0.5 per cent of them have 3 or more consecutive spontaneous abortions. This group of women are categorized as having " habitual abortion". More than 50 percent of spontaneous first trimester abortion specimens that undergo karyotyping are found to have a chromosomal abnormality, which is the major cause for spontaneous abortion. Karyotype studies of couples with two or more spontaneous abortions revealed in 2.78 to 3.4 per cent of the cases that one of the couples was a balanced reciprocal translocation carrier. By high resolution banding technique a slightly higher rate (4.76 per cent) was detected. 1328 persons (664 couples) referred to our Genetics Center with the hisorty of two or more consecutive spontaneous abortions for chromosome studies. We found major chromosome abnormalities in 35 cases (5.27 per cent). The most common type of abnormalities was translocation, in 7 cases the mosaicism of sex chromosome abnormalities has been detected. In one case Yq inversion, one case 46,XY male pseudohermaphroditism, and another single case of pericenteric inversion of chromosome No 1 were the final diagnoses. On mosaic cases the most common type, was the mosaic Turner syndrome (4 cases), followed by the mosaic Klinefelter syndrome with 2 cases. We found only 1 case with mosaic trisomy 13.
We were confronted with 68 cases that showed minor chromosome abnormalities (10.2 per cent). The most common anomaly was pericentric inversion of chromosome No.9 (17 cases).

P0363. Is tumourigenesis triggered by chronic inflammation?

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During the last years, investigators aimed at the identification of new dignostic and prognostic features of tumourigenesis by correlating cytogenetic data with the histopathological stage of tumour. Several tumours (e.g. esophagus, colon, lung) were characterized to be preceeded by chronic inflammation.
In an effort to evaluate if there was a relationship between chronic inflammation and tumour development we determined tumour indicating parameters like polyploidization, centrosome hyperamplification, multipolar mitoses and the state of p53 during inflammation in humans and mice.
In inflammatory tissue of human and rodent wounds we detected an increased tetra- and polyploidization rate, accompanied by centrosome hyperamplification in human wounds. Tetraploid cells seem to be advantageous during times of unfavourable circumstances, as they are during inflammation. Their number decreased at the end of the healing process during scar formation, coordinated by an intact p53.
In inflammatory tissue of the bronchus, we also detected tetra- and aneuploid cells as well as centrosome hyperamplification, both increasing with the grade of inflammation. Since tetraploidy is defined as an intermediate during tumourigenesis, a decrease of this population, which we could see after transient inflammation in the human wound, is necessary to prevent a malignant development. However chronic inflammation, as in the bronchus, promotes aneuploidization and malignancy because the unfavourable conditions are persistent and expose the cells to prolonged stress. We could also show, that an enhanced number of centrosomes plays a critical role during the process of segregating the chromosomes into normal euploid (diploid) cells as well as into aneuploid ones.

P0364. Detection of cerbB-2 gene amplification in bilharzial bladder cancer using fluorescence in situ hybridization

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Gene amplification are common in many different tumor types and may confer diagnostic, prognostic, or therapeutic information for patient management. Amplification and overstatement of the cerbB-2 gene occurs in different types of cancer. Fluorescence in situ hybridization (FISH) represents the newest methodological approach for testing for this genetic alteration.
In this study, FISH is used in a series of archival human bilharzial bladder cancer specimens to evaluate for the presence of cerbB-2 gene alterations in the most common malignant tumor in Egypt and some other countries. The study included 41 cases, 32 males and 9 females. Twenty-one cases had squamous cell carcinoma, 17 had transitional cell carcinoma, 2 had adenocarcinoma, and one case had undifferentiated carcinoma. After performing radical cystectomy to these patients, stage p3b was the commonest lesion being present in 22 cases, while p3a lesions occurred in 9 cases, p2 in 6, and p1 in one case. Pathologic tumor stage could not be assessed in 3 cases.

Pelvic nodal affection occurred in 12 out of 36 (33.3 %) in whom pelvic nodes were examined.
Our data demonstrate that 16 of 41 tumor samples (36.6%) show evidence of true cerbB-2 gene amplification. Of the remaining samples, 21 (53.4%) show no gene amplification and 4 (10 %) fall into the borderline category with a ratio between one and two cerbB-2 genes/cell relative to chromosome 17 centromeres. Our data indicate a possible role of the cerbB-2 gene in the development of aggressive behavior in bilharzial bladder tumors.

P0365. Micronucleus Frequencies in Buccal Mucosa,Urothelial Cells and Peripheral Blood Lymphocytes of Smokers.

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The micronucleus (MN) assay in human peripheral blood and exfoliated cells has been widely used to detect genotoxic effects of environmental mutagens, infectious agents and hereditary diseases. In the present study, we aimed to determine the genetic toxicity of cigarette smoking. MN assay was performed on buccal mucosa, urothelial cells and periheral blood lymphocyte samples obtained from 15 healthy male smokers (> 5 pack-years) and 15 male non-smoker controls who had not been exposed to any known genotoxic agent. It was found that, the mean value (+/- SD) of MN frequency in oral mucosa cells from smokers and controls were 1,208 ± 0,220 % and 0,264±0,105 %, in urothelial exfoliative cells 1,292±0,280 % and 0,120±0,088 %, in peripheral blood lymphocytes 1,534±0,234 % and 0,386±0,124 %, respectively. The mean MN frequencies in the buccal mucosa, urothelial exfoliative cells, and peripheral blood lymphocytes were significantly higher in smokers than in those of controls (p< 0,0005). Our data suggest that chromosomal damage in these tissues were induced by cigarette smoking.

P0366. Chromosomal aberrations in Cis and Ta bilharzial bladder cancer

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Carcinoma of the bladder is the most prevalent cancer in Egypt and most African countries.At the National Cancer Institute, Cairo, it constitutes 30.3% of all cancers.The median age at diagnosis is 46 years,with male preponderance of 5:1.It has several unique clinical, epidemiological, and histological characteristics suggesting that it is a distinct entity from bladder cancer seen in Western countries.Genetic alterations in bilharzial related bladder cancer have been studied infrequently, and specially in the advanced sittings i.e. T3 and T4 stages.The objective of this study was to extend establishing the base line cytogentic profile of this type of malignancy to carcinoma in situ stages. For this purpose, FISH was applied to interphase nuclei of frozen-stored samples with biotinylated repetitive DNA probes specific for all chromosomes to detect numerical chromosome changes in 25 patients presenting with carcinoma in situ of bladder. Twenty four cases had transitional cell carcinoma and one case had squamous cell carcinoma. Six out of 24 TCC cases had diploid chromosome count with all the probes. Numerical chromosome aberrations were detected in 18 cases (75%).In 8 cases, a loss of chromosome 9 was observed.In one case, an additional loss of chromosome 17 was detected.One case demonstrated a loss of chromosome 17, whereas another two cases showed a gain of chromosome 7. Loss of chromosome Y was observed in 9 of the 22 male cases studied (40.9%).The only case with SCC had normal diploid chromosome count with all the probes used. A theory of bilharzial bladder cancer pathogenesis is suggested.

P0367. Partial Deletion 18p in a Patient with Psoriasis Vulgaris

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Deletion 18p (18p-) is characterized by variety of clinical features including mental retardation, facial abnormalities and skeletal anomalies. Skin abnormalities are rarely reported. There are no cases of psoriasis vulgaris in patients with the 18p- syndrome. Psoriasis vulgaris is a chronic inflammatory skin disease that affects about 3% of the caucasian population. The psoriasis susceptibility loci have been suggested within many chromosomes (1p, 1q, 2p, 6p, 17q etc.). We describe the first case of psoriasis vulgaris in a male with 18p- syndrome. Examination at the age 27 years showed psoriatic red scaly patches on the skin of his head and forearms. He was moderately mentally retarded and had dysmorphic features: short stature, downslanted palpebral fissures, exophthalmus, prognathism, kyphosis, brachydactyly, micropenis etc. Cytogenetic analyses using GTG banding revealed partial deletion 18p in all cells studied. FISH showing two fluorescent signals with WCP18 DNA probe and one signal with 18p subtelomeric DNA probe on metaphases confirmed the finding. Karyotype is 46,XY,del(18)(: p11.23-qter). As psoriasis vulgaris has not been previously described in patients with 18p- syndrome, one might argue that they have not reached an age to be able to determine whether they are affected with psoriasis or not. Follow-up examination of these patients will be necessary. The coexistence of psoriasis vulgaris and 18p- in our patient is possibly an association, but it might also be a causal connection and may be helpful in the localization of an additional susceptibility locus for psoriasis in the region of 18p.

P0368. Case report of a de novo duplication 14q11.2-q21.2

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We present the case of a 10-month-old girl, second child of nonconsanguineous healthy parents, with developmental delay, growth retardation (height and head circumference 3rd percentile), several dysmorphic stigmata and severe West syndrome. Conventional chromosomal banding analysis led to the suspicion of one derivative chromosome 14. A whole chromosome paint for chromosome 14 gave a homogeneous staining, excluding an interchromosomal rearrangement. CGH revealed a gain of chromosomal material of the region 14q11-21. FISH analysis with region specific YACs confirmed the duplication. The duplication spans from 14q11.2 to 14q21.2. and accounts for approximately 16Mb. Chromosomal analysis of both parents showed normal karyotypes. Even a minor 14q duplication of one parent was excluded by FISH. There is no published case of proximal duplication 14q with exactly the same breakpoints. Thus, it is conceivable that not all clinical features of our patient are concordant to other patients with proximal duplication 14q. Especially West syndrome has yet not been described in this context.
Methodologically our case confirms that CGH is a useful tool in molecular cytogenetics particularly for the detection of small chromosomal rearrangements not concerning the subtelomeres for which a specific diagnostic is available.

P0369. The shape, length and banding pattern of human interphase chromosomes

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Interphase chromosomes analysed with currently available techniques do not present any recognizable structures such as bands, centromeres, telomeres, or specific shapes. Microirradiation experiments and molecular cytogenetic investigations with whole chromosome paints and region specific microdissection probes have confirmed a territorial organization of chromosomes in interphase nuclei. Until now, however, their structure is not well understood. Using laser scanning microscopic examination and the high-resolution DNA-based multicolour banding (MCB) technique, we have generated a banding pattern and have determined the length of human chromosome 5 in lymphocyte interphase nuclei, and in nuclei of HeLa cells arrested at different phases of the cell cycle. The shape and MCB pattern of chromosome 5 in interphase nuclei

is similar to that of metaphase chromosome 5 at all stages of the cell cycle. The length of the chromosome axis is comparable to that of a metaphase chromosome at a 600-band resolution. Therefore, the concept of chromosome condensation during mitosis has to be reassessed. Interphase chromosome banding can be used to identify chromosome aberrations and opens new fields in cytogenetic analysis.

P0370. Breakpoints in 17p13 associated with Asperger syndrome.

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Asperger syndrome (AS) is a severe developmental disorder characterized by major difficulties in social interaction with unusual responses to the environment similar to those in a mild form of autism. 0.3-0.6% of the population will meet the criteria of AS. The clinical features includes paucity of empathy, difficulties to form friendships with social isolation as a consequence. The syndrome also includes pedantic and monotonic speech and highly circumscribed interests in topics such as the weather and railway tables or maps.
We have identified two non-related patients with AS both with apparently balanced reciprocal translocations with breakpoints in 17p13. Cytogenetic analysis revealed *de novo* translocations t(13;17)(q14;p13) and t(17;19)(p13;cen) respectively.
Mapping the translocation breakpoints of the chromosomes 17p was performed by FISH. Chromosome 17p13 specific clones were hybridized to metaphase chromosomes of the two translocations. The results demonstrated the two breakpoints to be located within a region of 300 kb. The region is spanning 14 known genes.
PCR- amplified fragments derived from or flanking 5 of the genes was used as FISH-probes to perform a closer definition of the breakpoints.

P0371. Using chromosomal microdissection for production of specific molecular probes.

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The microdissection of chromosomes is the technique for production high specific molecular probes, which are necessary for diagnostic fluorescence in situ hybridization (FISH) with structural and numeral abnormal chromosomes, including marker chromosomes. The specificity of these probes is connected to identification of chromosomal aberration in precise clinical case, which diagnosis by using commercial probes is very expensive and time-consuming. To this time we prepared microdissection of marker chromosomes from human and translocated chromosome X from dog. We prepared also probes from X and Y bovine chromosomes for diagnosis of aberration of these chromosomes. All FISH experiments showed the hybridization signals and allowed for identification of studied cases. From our experience we know that microdissection of chromosomes is irreplaceable tool for obtain of probes more adequate for the specific clinical case than commercial probes.
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P0372. Micronuclei Induction in Rat Embryonic Blood Cells Following Exposure to Different Modes of Electromagnetic Fields

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In modern society, human population have been exposed to different modes of electromagnetic fields (EMFs). Many researchers have been conducted on whether cancer risks may be associated with such exposures. It has known that low frequency EMFs don't produce enough energy to damage DNA. However, epidomiological studies suggest that EMFs have been associated with increased incidence of cancer risk and must be considered as a potential genotoxic agent. In this study, genotoxic effects of different modes of magnetic fields were investigated by using the micronucleus assay. For this, rat embryos were exposed to different modes(5,10,20,30mGA) of EMF 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-1040 medium.The sample was directly processed for micronucleus assay.Results from control and experimental groups were compared statiscally.We did not observe any significant difference between the control and experimental groups (p>0.05),at these low modes of EMF. Further experiments will be carried out in order to examine the genotoxic effects of higher doses of EMF on rat embryonic growth and development.

P0373. A complex sex chromosome abnormality associated with short stature and hypogonadism

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Feminine hypogonadism is characterized by delay puberty, absent of the sexual characters and primary amenorrhea. In some cases these findings are due by gonadal dysgenesis which are based a numerical or structural chromosome anomalies.
We reported a 15 years 2 months old girl with proportionate short stature, thin body, delay puberty (primary amenorrhea, B1, PH3). Supplementary features were excessive pigmented nevi (on face and thorax), narrow, hyperconvex nails and thumbs with radial angulation. The pregnancy and delivery were uneventful and the proposita was born at term with 2300g weight. She is the first child of young healthy non-consanguineous couple. Both parents have healthy children from others marriages.
Sex chromatin showed two Barr bodies. Other investigations performed are showing a mean intelligence (IQ= 106), high levels of FSH and LH and the presence of uterus with bilateral polycystic ovaries (echographic exam of pelvis).
G-banded chromosomal analysis (with an average resolution of 450-550 bands) revealed a 46,XX/47,XXX/45,X/46,X, +mar karyotype. The patient combine clinical features from different gonadal dysgenesis (Turner syndrome, triplo X). The karyotype could explain miscellaneous features. The origin of marker presently being arranged for and results, if any, will be shown at the meeting

P0374. Investigation of chromosomal aberrations in hepatocellular carcinoma in Egypt by fluorescence in situ hybridization

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Hepatocellular carcinoma (HCC) is a very common and highly malignant tumor, associated mainly with chronic viral hepatitis, cirrhosis of any cause, aflatoxin exposure and ethanol consumption. Cytogenetic analysis on HCC has been limited because of poor hepatocyte growth in vitro. Conventional cytogenetic studies have demonstrated frequent abnormalities of specific chromosomes in hepatocellular carcinoma. Molecular cytogenetic approaches have applied only rarely in the characterization of hepatocellular carcinoma (HCC). The main aim in this study was to evaluate genetic aberrations of different chromosomes in HCC.
The study included 30 patients with hepatocellualr carcinoma who have been diagnosed and treated at National Cancer Institute, Cairo University during the period 1997-1998. The charts of the patients were reviewed to retrieve their clinico-pathologic data. They were 20 males and 10 females with a M/F ration of 2. Their ages ranged between 14 years and 80 years (median 55 years).
Interphase cytogenetics by fluorescence in situ hybridization with the use of a panel of centromere-associated DNA probes for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were performed on paraffin-embedded HCC specimens. Numerical abnormalities of chromosomes 1, 4, and 6 were found in 7, 15 and 12 cases, respectively. Trisomies of chromosomes 8 and 9 were found in 6, 9 cases respectively. Gain and/or loss of more than one chromosome were detected in 27 of 30 cases. Gains and losses of DNA found in

this study probably involve oncogenes and tumor suppressor genes that play a role in the puzzle of hepatocarcinogenesis.

P0375. The effects of diagnostic ultrasound exposure on the meiotic prophase in rats oocytes.

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Over the past 30 years ultrasound has become a widely used tool in obstetrics diagnostic. The safety of obstetric ultrasonography was proved with standard teratologycal methods. Recently a number of biological effects have been observed following diagnostic ultrasound exposure in various experimental systems (Tarantal, Hendrickx, 1989; Jensh et. al, 1995; Newnham et. al., 1993). Taken into account a widespread application of ultrasound in prenatal medicine and obstetrics its effect on oogenesis in mammalian fetuses deserves special attention.

The subjects were 19 albino rats. The proportion of meiotic prophase stages in the fetal ovaries on the 21st day of development were studied after 30 minutes exposure to diagnostic ultrasound (intensity <100mW/cm2, frequency 5MHz) of female rats on the 17th day of pregnancy (group 1). The control pregnant rats were assigned to two other groups: group 2 - the sham-irradiated group that was treated in the same way except for irradiation (n=5); group 3 - untreated group (n=5). Most cells in all groups (56,44±8,33 - in group 1; 63,66±6,03 - in group 2; 69,68±10,8 - in group 3) demonstrated zygotene-pachytene figures. No differences were found in distribution of meiotic prophase stages in the groups. Our data demonstrate that diagnostic ultrasound exposure has not significant influence on the developmental schedule of oocytes maturation.

P0376. Analysis of meiotic prophase in normal and abnormal human female fetuses

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Mammalian oocytes at meiotic prophase stage demonstrate a remarkable sensibility to different mutagenic factors (Kolomiez et. al., 1992), therefore the investigation of exogenous and endogenous factors effect on human germ cells dynamics is of specially importance.

The 19 human female fetuses on 19-26 weeks of gestation were assigned to 4 groups respective to abortion reasons. The distribution of meiotic prophase stages in the oocytes was studied on 76 cytogenetic preparations.

In fetuses on 19-20 weeks of gestation (n=12) meiotic figures were represented by predominantly zygotene stage cells. In spontaneous abortion the amount of diplotene-dictiotene cells was statistically lower (t=2,94 P>95%) compared to the control group (social reasons abortion).

During the 23-26 weeks of gestation in malformations fetuses with normal karyotype and (n=4) dictiotene stage predominate, while in aberrant karyotype group (n=3) the amount of cells on this stage was 10 times less.

The decrease in amount of cells at the end of prophase 1 can be attributed to partial degeneration of oocytes during the preceding stages.

The differences in distribution of oocyte meiosis stages in the embryos of the same age suggest the existence of different factors that influence on meiotic prophase in human oogenesis

P0377. Balanced chromosomal anomalies found in persons with reproductive failures

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Sometimes in the field of human reproduction we forget about the necessity of cytogenetically examination of infertile couples. We found some cildless couples that have already been treating their infertility for years. In three of such couples we identified one partner as a carrier of a constitutional chromosomal anomaly. From them two cases were certified as familial rearrangements. First, a pericentric inversion of chromosome 5 in a normospermic man whose wife, after seven spontaneous abortions, finally gave birth to a healthy boy who inherited the same karyotype from his father. The second one, a maternal translocation t(13;18) in a woman detected after eight years period of sterility and two spontaneous abortions. Another balanced translocation t(5;13) without familial data was found in a normospermic man whose wife had four repeated unsuccessful pregnancies. When the pacient learnt about the diagnosis he did not want to cooperate any longer, so we could not contact other members of his family. Our results point out the value of the cytogenetically screening of the couples with reproductive failures as well as the emotional and familial aspects of the genetic counselling.

P0378. Molecular cytogenetic characterization of disseminated tumor cells in renal cell carcinomas

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Bone marrow is the most important secondary organ for the dissemination of epithelial cells in tumor patients. Several recent studies demonstrated that the detection of such disseminated cells may represent an independent prognostic factor in several tumor entities. However, in urologic tumors the role of disseminated epithelial cells has up to date not been established. Furthermore, a detailed characterization of the genome of these rare events remains an arduous task. The aims of our study include the development of new molecular cytogenetic methods for a detailed characterization of disseminated cells and their application on bone marrow of patients with urologic tumors, such as renal cell carcinomas and bladder cancer.

Our molecular cytogenetic strategy employs in a first step an enrichment of disseminated cytokeratin-positive cells using established protocols (e.g. magnetic beads). In a second step, disseminated cells are analyzed either by multicolor interphase-FISH and/or by single cell CGH. Multicolor interphase-FISH is done by using simultaneously at least seven different DNA-probes, each labeled in a different color.

RCC was chosen as a model tumor entity for two reasons: firstly, our knowledge about cytogenetic rearrangements in this tumor entity is fairly advanced. Secondly, chromosomal rearrangements have a limited complexity .

First applications of this strategy demonstrating the feasibility for a high resolution characterization of the genome of rare cells will be shown. This strategy should allow to gain new insights into genetic changes involved in tumor progression and in early dissemination. The application of these methods is currently also extended to bladder cancer.

P0379. Karyological analysis of immature germ cells in sperm from men with impaired spermatogenesis.

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The diagnosis of male infertility is based on analysis of ejaculate and testis biopsy. Due to definite limitations of testis biopsy analysis of ejaculate is usually more preferable. Semenograme allows to obtain information about mature germ cells: concentration, motility and morphological characteristic of spermatozoa. However, more detailed information about spermatogenesis might be obtained by analysis of ejaculated immature germ cells using the method called Quantitative Karyological Analysis of Immature Spermatogenic Cells (QKAISC) (Kurilo,1993). This method permits to determine the relative portion of germ cells at different stages and the stage of spermatogenesis block.

The samples of semen from 4 control subject and 25 patients with impaired spermatogenesis were analysed using QKAISC technique. The partial block of spermatogenesis at the prepachytene stages was detected in 2 patients with 47,XXY, but in patient with 46,XY/47,XXY spermatogenesis block was detected after MI division. The partial arrest of spermatogenesis at the prepachytene, pachytene and diplotene stages was detected in 2 patients with 46,XY t(9;13) and 46,XY t(Y;5). In 8 46,XY patients with azoospermia the increased number of degenerated spermatogeneous cells and somatic cells was detected. In 5 46,XY patients with oligoastenoteratospermia and oligoastenospermia the partial block of spermatogenesis was detected after MI division with increased number of degenerated

spermatogenic cells. There were no difference between control subjects and patients with normal and minor impairments of semen characteristics. These results demonstrate the significance of QKAISC technique in performing on complex examination of ejaculate from patients with infertility.
Kurilo et al. Probl. Repr.(rus) 1995. v.3. p.33

P0380. Interstitial deletion in 10q11.2 : a nonpathogenic euchromatic deletion

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Euchromatic autosomal imbalance detectable at the cytogenetic level is usually associated with mental retardation and congenital abnormality. However, exceptional families have been reported in which cytogenetically visible deletions are segregating without detectable phenotypic effect. We report on a family in which a young man was referred for chromosomal analysis for infertility associated with azoospermia. A complex chromosomal rearrangement was observed, comprising a reciprocal translocation with a deletion : 46,XY,der(10) del(10) (q11.2q11.2) t(4 ;10)(p15.1 ;q11.1). Blood from his brother and their mother was cultured and the same chromosomal abnormality was found in both. FISH study with specific whole chromosome painting probes and comparative genomic hybridization were performed both in the index case and his relatives, confirming the complex rearrangement. Reciprocal translocations may be associated with male infertility, as an insurmontable obstacle to cell division in the spematocyte, resulting in azoospermia.(oogenesis is apparently less vulnerable). To our knowledge, only one case of this deletion have been reported in the literature, without phenotypic consequences. Based on these data, deletion of band 10q11.2 appears to have no phenotypic consequences.

P0381. Sex reversal secondary to Xp functional disomy including DAX1 gene by t(X;Y)(p21.2;p11.3)

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Translocations involving the short arms of the X and Y chromosomes are extremely rare. They result from a recombinaison in the paternal germline. The most frequent are reported in XX males and rarely in XY females, resulting from SRY gene transfer, leading to complete sex reversal. Male-to-female sex reversal has been also observed in individuals with partial duplication of Xp and an intact SRY gene. The dosage sensitive sex-reversal is due to duplication of the DAX1 gene in Xp21.2. We describe a 7 month-old phenotypic female child with severe psychomotor retardation, growth retardation, dysmorphic features, cleft palate, cardiac and cerebral anomalies. The patient's karyotype was 46,X,+ mar in all cells examined. The karyotype of parents were normal. FISH techniques provided evidence that the derivative chromosome was a der(Y) resulting from the transposition of Xp material, including Xp21, onto the terminal short arm of an Y chromosome. No deletion of the Y chromosome was detected. The child's karyotype was: 46,X der (Y)t(X;Y)(p21.2;p11.3). Abnormal clinical findings of our patient is due to funtional disomy for Xp21.2-pter segment, because the Xp translocated portion is active. Her complete sex reversal results from the presence of two active copies of DAX1 gene. The phenotype of our patient is similar to previous reports of genetic males carrying a partial duplication of Xp. To our knowledge, this is only the second report of a t(X;Y) resulting from the transposition of Xp to the short arm of the Y chromosome. Genes involved in human sex determination are discussed.

P0382. Are There Interchromosomal Effects of Chromosomal Rearrangements on Occurrence of Aneuploidy in Sperm Nuclei of Carriers?

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Translocation carriers have been considered as reproductive failures, either due to spermatogenetic arrest or to unbalanced progeny. During the meiosis, both translocated chromosomes and their normal homologues can lead to unbalanced patterns of segregation for these chromosomes. Analysis of live born offspring or fetuses does not provide accurate information about meiosis segregation products because of early missing of abnormal conception. However, it has been postulated that there is an increased frequency of aneuploidies involving unrelated to the translocation, termed as interchromosomal effect. Therefore, in this study, we aimed to present our primary findings for evaluation of interchromosomal effects of different rearrangements, such as different balanced reciprocal and Robertsonian translocations and inversion, on occurrence of aneuploidies of sperm nuclei of carriers. Interphase sperm-fluorescence in situ hybridization (FISH) analysis was performed by using chromosome specific DNA probes that were not involving in those rearrangements

P0383. High quality BAC FISH probes for the detection of chromosome imbalances

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Conventional fluorescence in situ hybridization (FISH) is a useful tool for the detection of human chromosomal abnormalities in prenatal and postnatal diagnostics. The quality of DNA probes contributes to the signal detection and is important for a confident interpretation of FISH results. We have developed high quality FISH probes suitable for the identification of the major trisomies (21, 13, 18) as well as common structural rearrangements such as deletion of 22q11. Most of our BAC clones were isolated by using the REPuter program which allows us to visualize distributions of exact and degenerate repeats with a minimal length of 20 bp. Regions with high gene content and relatively low repeat density were selected and primers were designed for the identification of BAC clones. Furthermore, for improvement of the FISH signals we have assembled BAC contigs and used these as complex probe mixtures. The BAC clones were mapped to different regions of chromosome 21 including the centromeric region of 21q11, the Down syndrome critical regions at 21q22, and subtelomeric region of 21q22.3. Several DNA probes were isolated in the region of 13q32, 18q21, 22q11 and subtelomeric region of 22q13. The probe set extremely facilitates the detection of specific chromosome imbalances on uncultured amniotic fluid cells and metaphase chromosomes and is greatly useful for the identification of partial trisomies, partial monosomies and interstitial deletions.

P0384. Gonadal dysgenesis. Presentation of 3 cases and report on the database of a Paediatric Endocrine Unit in Hungary.

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Gonadal dysgenesis encompasses a heterogeneous group of different chromosomal, gonadal and genital abnormalities characterised by the presence of dysgenetic testes and/or streak gonads, persistence of Müllerian duct structures and a variable degree of genital ambiguity or female phenotype. Clinical, pathological, hormonal and genetic aspects of 24 patients with complete or partial gonadal dysgenesis have been studied. Three patients had complete (pure) gonadal dysgenesis (karyotype: 46,XY). The karyotype of the 21 patients with a clinical diagnosis of partial gonadal dysgenesis (PGD) was 45,X/46,XY (n=10), 46,XY (n=6) or others (n=5). The types of PGD, based on pathological findings of the gonads, were mixed gonadal dysgenesis in 5 cases, dysgenetic male pseudohermaphroditism in 6 cases, bilateral gonadoblastoma in one case (no histological data are available in 9 cases). Genital phenotype was predominantly male (n=8), ambiguous (n=9) or predominantly female (n=4).
We present 3 new cases with testicular intersexuality. The cytogenetic investigations were carried out on both peripheral lymphocyte cultures and on buccal cells by GTG and FISH technics. The karyotype analyses of two patients with partial gonadal

dysgenesis showed 45,X/46,XY mosaicism in different proportion of tissues, while the third case proved to be 46, XY/47, XYY mosaic with genital ambiguity. The phenotype and genotype correlations and hormonal results are discussed.

P0385. Segregation of Pierre Robin Sequence with a 2;17 (q32;q24) Translocation

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The Pierre Robin Sequence (PRS) consists in the association of micrognathia, glossoptosis and cleft soft palate. PRS may present as an isolated (nonsyndromic) feature, generally on a sporadic basis, or may aggregate with additional findings that together define a syndrome, which in turn may itself be found to segregate in the family.

Although no genetic locus is known for nonsyndromic PRS, genetic factors are thought to play a role in this functional and morphological entity. The 2q32 region has been specifically associated with isolated cleft-palate.

We present a father and a daughter carriers of a balanced translocation between chromosomes 2 and 17 (2q32;q24) and with the phenotipic manifestations of the PRS. The paternal grandparents were studied and neither of them presented the traslocation nor the PRS.

HYPOTHESIS: The case presented here suggests the existence of a genetic base not only for the cleft-palate feature but for the nonsyndromic PRS located on 2q32. The absence of other phenotipic manifestations rather ssuggests the idea that in this family there is not a microdeletion but a breakage point in the possible “candidate gene”.

P0386. Identification of marker chromosomes using microdissection and FISH

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Micro-FISH is a technique that comprises the physical dissection of a GTG-banded chromosome (part), followed by a degenerate oligonucleotide primed-polymerase chain reaction (DOP-PCR) to amplify the dissected chromosomal material, labelling of the PCR product and subsequently reverse painting. In clinical cytogenetics micro-FISH can be used to characterise marker chromosomes, (de novo) unbalanced translocations and complex chromosome rearrangements.

We present four cases with an unbalanced karyotype due to the presence of (DA/DAPI and NOR negative) marker chromosomes. In a 8-year-old not mentally retarded boy, referred for cytogenetic examination because of growth retardation, a mos 47,XY,+mar[75]/46,XY[25] karyotype was determined. Micro-FISH showed that the minute marker contained the centromere of chromosome 8. Chromosome analysis in a 26-year-old female showed an extra marker chromosome in 50% of her lymphocytes. She was referred because of mental retardation, dysmorphism and obesity. The marker chromosome contained chromosome region 14q10→14q12. The third patient was referred for chromosome analysis at the age of 10 years because of growth retardation. She appeared to be carrier of 2 different marker chromosomes. At the age of 26 years chromosome analysis was repeated. In 47% of the analysed cells both markers were present, in 7% a small marker and in 29% a larger marker chromosome was found. Micro-FISH disclosed that one marker contained chromosome region 19q10→19q13.1, the other marker contained chromosome region 20p10→20p11.2. The fourth marker chromosome was present in 25% of amniotic fluid cells of a woman referred because of advanced maternal age. The contents of this marker chromosome was region 19p10→19p13.1.

P0387. A familial translocation t(3;5) identified by chance in a case of essential thrombocythemia (ET)

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In this paper we report a familial translocation t(3;5)(q26;q21) found by chance in a 72 year old female at the moment of the diagnosis of ET. Because the patient had an excellent treatment outcome and rapidly achieved hematological remission we supposed that this constitutional translocation might not be involved in ET pathogenesis, but rather in the reproduction. This is the reason why we extended the cytogenetic investigations in the patient’s offspring: her 52 year old only daughter and two grandchildren (a boy aged 22 and a girl aged 18). All of them are phenotipically normal including hematological values. Using peripheral blood samples for chromosomne slides and GTG-banding followed by FISH with telomere probes and whole chromosome painting (Appligene Oncor CP5406G, CP5410R and Cytocell, PCM 356) we identified the same translocation t(3;5)(q26;q21)in proband, her daughter and granddaughter, but not in the male offspring. The maternal inheritance of such a large translocation in three generations without striking reproductive failures is unexpected taking into account the involvement of chromosomal rearrangements in meiotic segregation of unbalanced gametes. In order to have a better and precise characterization of the breakpoints, extensive studies are needed. Molecular description of genomic region flanking the 3q and 5q breakpoints will help us to understand the relationship between this structural rearrangement and proband’s hematologic disorder (ET), if any.

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P0388. A Family With Reciprocal 4;7 Translocation

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There are reports on balanced translocations of chromosomes 4 and 7 with different chromosomes with various breakpoints in the parents of abnormal offsprings with unbalanced karyotypes, but we found no reports of t(4;7)(q31;p22) in our search of literature.

We present a family that have a balanced reciprocal translocation between chromosomes 4 and 7.

The proband who was 20 days old, was referred to our department because of clitoris hypertrophy. She had no other abnormalities. Her Na^+, K^+, and 17 α OH progesteron levels were, 139 (N:135-145), 5.5 (N:3.5-5), and 16.8 (N:1.05-40.41), respectively. Her father was 37, and her mother was 35 year-old at the time of delivery. The marriage was non-consanguineous. They had a healthy daughter who was 9 year-old. When the proband was reexamined after 6 months, her genitalia was appeared to be normal.

Cytogenetic analysis on peripheral blood cultures of the proband by GTL banding revealed 46,XX,t(4;7)(q31;p22) chromosome constitution. Then we performed cytogenetic analysis on the parents and the sister. Proband’s mother had 46,XX karyotype but we found 46,XY, t(4;7)(q31;p22) in her father and 46,XX,t(4;7)(q31;p22) in her sister. Then two sisters of the father were studied and revealed 46,XX, t(4;7)(q31;p22) karyotypes.

P0389. Using of dual-color in situ hybridization for detection of numerical abnormalities in uncultured and cultured leucocytes of radiochemical industrial workers

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As a rule the numerical chromosomal aberrations are not taken into account in the genotoxicity testing. However, it is possible that many harmful substances can cause this type of chromosomal alterations. Fluorescence in situ hybridization (FISH) is a powerful technique that allows numerical chromosome aberrations (aneuploidy) to be detected in interphase cells. We used dual-color FISH for detection of aneuploidy in peripheral blood leucocytes of 15 men – radiochemical industrial workers, contacted with complex of radioactive and chemical substances. Analysis of hypo- and hyperploidy was carried out for three chromosomal pairs: 7/12, 11/16 and X/Y using digoxigenin/biotin labeled probes. We found

significantly increased frequencies of numerical chromosome abnormalities in uncultured leucocytes of the exposed workers compared to the unexposed controls (10 individuals) for nullisomy Y-chromosomes only. However significant differences were found in cultured lymphocytes for hypodiploidy of chromosomes 11, 12 and hyperdiploidy of chromosomes X and 12. Moreover, a tendency to higher values was observed for hypodiploidy 16 and total frequency of numerical abnormalities in cultured lymphocytes. We conclude that agents of the radiochemical industry can induce aneuploidy in leucocytes and cause the premutagenic lesions, which are expressed during the cultivation of lymphocytes in vitro. It is possible, that some chromosomes have larger liability to nondisjunction and loss. Our data suggest that analysis of numerical aberrations must be carried out in addition to standard cytogenetics tests.

P0390. Inverted duplications associated with terminal deletions : the phenotypic impact of this newly recognizable rearrangement.

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Distal inverted duplications associated with a terminal deletion represent a newly recognizable category of chromosome alterations. Genotype-phenotype correlations are unclear.
We observed a dup/del rearrangement in 6 patients presenting with MCA/MR syndrome. It involved chromosomes 4p (3 patients), 5p (one patient), 1q (one patient) and 19q (one patient). The duplication was detected by conventional cytogenetics, the deletion by molecular probes only.
Chromosome 4p. Both the duplication and the deletion did differ in size in individual patients. The deletions, spanning 3.4, 10 and 12 Mb on 4p16, respectively, encompassed the Wolf-Hirschhorn syndrome critical region" (WHSCR)in each occasion. Clinically, all patients presented with a WHS phenotype.
Chromosome 5p. The 5p13.3-p15.1 region was duplicated, the deleted 5p15.1-pter segment was demonstred by FISH to include the "cri du chat" syndrome critical region. The patient presented with a "cri du chat" syndrome phenotype.
Chromosomes 1q and 19q. A very distal duplication was observed in these cases, affecting the 1q42-q44 and 19q13.2-q13.4 regions, respectively. Accordingly, a very small deletion, just including the subtelomeric region, was detected. Clinical signs in both patients were consistent with the respective partial trisomy syndrome phenotype.
We observed that, whenever the deletion included critical regions with strong pleyotropic effects, such as the WHSCR and the "cri du chat" syndrome CR, clinical signs were consistent with a "deletion" phenotype only. A partial trisomy syndrome phenotype was observed in cases with a small subtelomeric deletion.
A proper assessment of the deletion is recommended in dup/del rearrangements.

P0391. Mosaic Trisomy 7; An Age Related Or Tissue Specific Finding In Normal Human Tissues

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Mosaic trisomy 7 has been found in a variety of tumors, non tumorous lesions, and even apparently normal tissues. Although characteristic in some condition, the meaning and pathological relevance of trisomy 7 is still unclear. Recent investigation has given rise to the question, whether somatic gain of chromosome 7 is rather related to age than to a specific disease.
By FISH we analyzed the chromosome 7 copy number in blood lymphocytes of 30 healthy individuals with normal conventional karyotype selected for three different age groups. FISH was performed as cohybridization of centromeric probes for chromosome 7 and 10; 1000 interphase nuclei each were analyzed.
Furthermore to identify a potential tissue specific increase of trisomy 7 in a group of 12 old individuals (> 80 years) 200 interphase nuclei of three different tissues (blood lymphocytes, hair root , and mucosal cells) each were analyzed comparatively by FISH.
In blood lymphocytes between all groups for the analyzed chromosomes revealing trisomy rates of below 0,5%, respectively, no significant differences were evident; thus an age related increase of trisomy 7 could not be verified for this tissue. In the intraindividual comparison of cells from different tissues , however, while blood lymphocytes and hair root cells showed similar low trisomy 7 rates (on the average 0.8%) in all cases, a variable but significant increase of mosaic trisomy 7 up to 11,5% was identified in mucosal cells of several individuals. An age dependence of the observed tissue specific amplification of chromosome 7 has to be investigated.

P0392. Very large pericentric inversion of chromosome 4 giving rise to a rec(4)chromosome and Wolf-Hirschhorn syndrome.

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We report a case of Wolf-Hirschhorn syndrome in a 1.5-year-old boy born to phenotypically normal parents. Cytogenetic analysis revealed a deletion at 4p15.3 in the boy and a large pericentric inversion of chromosome 4 in the father. The family history was unremarkable. The boy had been born at term with low birth weight, 1700g.
Our examination at the age of 2 weeks showed marked growth deficiency, microcephaly, hypertelorism, strabismus, epicanthal folds, prominent glabella, cleft lip and palate, downturned "fishlike' mouth, micrognathia, dysplastic ears, left simian crease and hypospadias. He also had a cardiac defect (pulmonary stenosis). One year old, he showed profound mental retardation and severe seizures that were difficult to treat. The low birth weight and the clinical signs represent typical Wolf-Hirschhorn syndrome.
Standard cytogenetic analysis from peripheral blood lymphocytes indicated a 4p deletion, karyotype 46,XY,del(4)(p15.3). The mother's karyotype was normal (46,XX), but the father demonstrated a large inversion of chromosome 4, karyotype 46,XY,inv(4)(p15.3q35). We then confirmed in the proband, using FISH-analysis, submicroscopic distal 4q trisomy(q35-qter) in addition to the terminal 4p deletion(4pter-4p15.3).
FISH karyotypes:
father: fish inv(4)(p15.3q35)(D4S2930/D4S3186+mv::D4Z1+, D4S96+mv)
proband: fish der(4)(qter-q35::p15.3-qter)(D4S2930/D4S3186+mv::D4S96-, D4Z1+, D4S2930/D4S3186+st)pat
Distal 4q duplication is usually not associated with a distinct clinical phenotype, and therefore the predominance of the Wolf-Hirschhorn phenotype was to be expected in this patient. Semicryptic or cryptic large pericentric inversions represent an important cytogenetic mechanism that can give rise to terminal deletion/duplication syndromes. Prenatal diagnosis must be considered in these cases because of the possibility of recombinants, here, dup(4)(p15.3-pter) and del(4)(q35-qter).

P0393. The collection of DNA probes for prenatal, postnatal and preimplantation diagnosis by FISH

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Creation of representative DNA probe collection is still actual problem in laboratories of Central and Eastern Europe. Availability of different DNA probes could help in introduction of FISH technology in many laboratories, and, therefore, increase the efficiency of cytogenetic diagnosis. We have identified a large set of original cloned DNA sequences, which could be used as DNA probes in molecular-cytogenetic studies. Alphoid plasmid and cosmid clones, containing repetitive elements, hybridized to pericentromeric regions of most part of chromosomes were identified and tested in clinical-cytogenetic studies (Vorsanova et al., 1986, 1991, 1996; Soloviev et al.,1993,1994,1995). We have analyzed large-insertion PAC library, containing more that 110000 genomic clones. We have detected more than 1600 centromeric and 600 telomeric PAC clones. 350 centromeric and 320 telomeric PAC clones were initially analyzed by FISH. Large insertion clones from total genomic human PAC library were identified for most part of telomeric regions. DNA probes with high potential for diagnosis of common human aneuploidies,

involving chromosomes 21, 13, 18, X and Y were carefully selected in cytogenetic studies. DNA probes for centromeric and telomeric chromosomal regions are usefull both as markers for cytogenetic diagnosis and as tool for detailed analysis of numerical and structural chromosomal aberrations in pre-, post- and preimplantation diagnosis, including the analysis of fetal cells .Supported by grant COPERNICUS-2.

P0394. Peculiarities of late replication of chromosomes of human fetal and chorionic villi cell at 7 and 12 weeks of gestation.

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The pattern of late replicating metaphase chromosomes from 24-hours cultures of CVS and tissues fragments from five human embryos at 7 and 12 weeks gestation was studied. BrdU was added by impulses through 2 hours. Monoclonal anti-BrdU antibodies FITC - conjugated goat anti-mouse antibody were used to identify the BrdU-labeled sites.

Metaphase spreads with markers of late replications were chosen for analyses. The intertissue differences in initiation and termination of replication in pericentric heterochromatin of chromosomes 1, 9 were shown in chorionic villi and embryonic cells. Pericentric heterochromatin of chromosome 16 was shown to be latest replicating segment in both tissues compared to pericentric heterochromatin of chromosomes 1, 9.

At 7 weeks of gestation some G-bands of chromosomes 1, 2, 3, 4, 5, 9, 11, 13, 14, 16 replicated simultaneously with pericentric heterochromatin of chromosomes 1, 9, 16 in chorionic cells, while in embryonic cells they replicated earlier than pericentric heterochromatin.

At 12 weeks of gestation however these segments replicated simultaneously with pericentric heterochromatin of chromosomes 1, 9, and 16 in both tissues. These data suggest that changes in of replication pattern of heterochromatin regions probably reflect differences in their functional status in embryonic and extraembryonic tissues at different stages of embryonic development.

P0395. Rare cytogenetic Klinefelter syndrome variant

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A 49-year-old male with hypogonadism and gynecomastia was found to have a 46,XX karyotype with additional chromosomal material on the short arm of a chromosome 15. C-banding and NOR-banding as well as fluorescence in situ hybridization with chromosomes 15 and Y centromeric probes, a Y chromosome short arm unique locus probe and a Y chromosome long arm heterochromatin probe were perform to further characterize this extra material. It was found to contain the Y chromosome short arm and centromere. This case represents a rare cytogenetic Klinefelter syndrome variant, with a diploid complement and a stable dicentric derivative chromosome from an unbalanced translocation involving the long arm of the Y chromosome and the short arm of a chromosome 15.

P0396. Molecular studies of small marker chromosomes in patients with 45,X/46,X,+mar mosaicism

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We have studied the small marker chromosome in four patients with mosaic karyotype 45,X/46,X+mar. Male patients were referred for azoospermia and female for primary amenorrhea. We used fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) to determine the origin and structure of the marker chromosome. FISH studied with SRY/CEPX probe (Vysis) confirmed the Y origin of the marker and detected that four patients showed double SRY signals on both ends of abnormal Y chromosome and one male patient had only one SRY gene. PCR analysis using primers for 11 loci along Y chromosome including SRY, RPS4Y, DYS14 (Yp), DYZ3 (Ycen), sY84 (AZFa), sY129, sY134 (AZFb), sY156, sY254, sY255 (AZFc, DAZ gene) and DYZ1 (Yqh) was investigated. Two male patients were the Yq breakpoint to the region between AZFa and AZFb. In female patient, the genes from SRY to AZFb were positive. In other two patients there was a failure to detect the alpha-satellite (DYZ3) that is an the integral part of the centromere. One of them was the Yp breakpoint between DYS14 and DYZ3, and the other having one SRY signal carried the Yp (SRY, RPS4Y, DYS14) and Yq (sY84, sY129, sY134) except DYZ3 unexpectly. It is assumed that the deletion of distal Yq euchromatin playing an important role in the spermatogenetic process lead only to azoospermia.

P0397. The chromosomal abnormalities value in primary amenorrhea etiology.

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Cytogenetic analyses were carried out on 54 cases of primary amenorrhea, using peripheral blood lymphocytes, GTG and CBG-banding. The monosomy X occured in 49 patients with Turner syndrome(TS). Chromosomal sexual assignment was established as 46,XY in three phenotypically females. One of them revealed gonadal tumor when she underwent gonadectomy at the age of 21. The involvement of autosomes in the ethiology of primary amenorrhea came into discussion in two cases;the karyotyping showed the folowing cellular mosaics:[46,XX,+18pter(80%)/45, XX,-14,+18pter(20%)] and [45,X0(60%)/46, X0,mar(?)(40%)], respectively.

At the time patient 1 reffered, she had hormonal-induced intermittent, irregular menses. We attempted to find correlation between the constitutional +18pter and the observed resistance to hormone-replacement therapy. Normal X chromosome pattern was noted both in hypodiploid and diploid cells. Neither the cryptic sex chromosomal abnormalities nor the origin of the 18pter extra material from chromosome 14 are excluded. Regarding patient 2, we mention that monosomy X was present in all 200 examined metaphases, strongly suggesting TS as cause of primary amenorrhea, but the patient lacked Turner stigmata. Moreover, the diploid cells exhibited a marker that appeared to be a dicentric derived from a translocation between 21 and 20 chromosomes. The cytogenetic approach proved once again its value in diagnosis of primary amenorrhea. Our study has to be extended at molecular level, in order to define accurately the provenience and the roles of the markers, especially those derived from autosomes.

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P0398. Result of Cytogenetic Study of 850 Bone Marrow Samples

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More than 850 bone marrow samples have been analyzed in our center during the past five years. Among 850 patients, 486 (57%) were referred for malignant disorders and 131 cases (15%) for non-malignant disease. 233 casses (28%) didn't have any primary diagnosis at the time samples were taken of which 169 belonged to the first 500 samples. Of the non-malignant cases, 87 were referred for anemia, 43 pre-transplant, 44 post-transplant, and 44 for post transplant study of a malignant disorder. The probable clinical diagnoses for the malignant cases were as follow: 164 (34%) for ALL, 135 (28%) AML, 123(26%) CML, 23(5%) MDS, 21 (4%) lymphoma, 6 (1%) Multiple Myeloma, 6 (1%) MPD and 8 (1%) others.

Chromosomal aberrations were detected in 73 (50%) of the 145 successful cultures of patients without any diagnosis, 69 (54%) of 128 cultures with diagnosis of ALL, 68 (55%) of 123 AML patients, and 80 (73%) of 109 patients suspected for CML. Among 22 conclusive MDS cases, 11 (50%) patients had some chromosomal change, ten (50%) out of 20 lymphoma cases and 3 out of 6 MPD and none of the 4 Multiple myeloma had chromosomal changes.

Among 88 post-transplant cases, 44 (50%) were malignant and 44

(50%) non-malignant. 72 (82%) patients showed donor cell line, 8 (9%) patients showed the recipient cell line and another 8 (9%) patients showed chimerism of donor and recipient cell lines.

P0399. Evidence for nonhomologous meiotic co-orientation (NMC) in man

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Homologous Robertsonian translocations/isochromosomes represent unique model for studying chromosome behavior in the absence of homologous pairing and recombination. Though the number of published cases is small, and the only data available is the sex of the offspring of the dup(21q) carriers, some conclusions on the segregation pattern of both rearranged chromosomes and gonosomes can be made. It was found that 7 male carriers of dup(21q) fathered 13 children with Down syndrome of known sex. 12 of them were males, differing from 1:1 ratio (p=0.0017, binomial test). There was no significant male predominance in cases of maternal transmission (23 males, 16 females). This finding is considered as a direct evidence for NMC of the dup(21q) and X-chromosome. NMC, proven for Drosophila [Grell, 1971], was proposed to explain male excess in Down syndrome (DS) with paternally derived trisomy [Kovaleva NV.Genetika(Russ)1992,28:5-15]. NCM of chromosomes 21 and X would result in production of different gametes: 23,X, 23,Y, 23(+21,-Y), 22,XY(-21), 22,0(-Y), 22,X(-21), 24,Y(+21), 24,XY, also explaining the occurrence of gonosome aneuploidy and double aneuploidy of paternal origin. Further data supporting the NMC hypothesis come from studies on sperm in healthy and infertile men [Griffin et al,1996; Baumgartner et al,1999], in fathers of patients with aneuploidy [Blanco et al,1998; Soares et al,2001] and in carriers of balanced translocations ("interchromosomal effect") [Morel et al,2001; Pellestor et al, 2001], together with data on elevated risk of progeny with autosomal trisomies in carriers of sex chromosome anomalies (Nivelon-Chevallier et al, 1988; Tarani et al, 1998; Hennebicq et al, 2001).

P0400. One karyotype with two features,a report from Iran.

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The index case was a female with a lichenoid lesion on her face. She reffered to our center for G banding karyotype because her sons were going to marry and she was worried about her son's future. She was an educated woman and a laboratory technician and there wasn't any sign of mental retardation. Her father had 4 children from her mother and four children from another wife. She had a nephew from her brother and a neice from her sister in law. Both children were mentally retarded and translocation (2,11) were found in the both children.
G banding method was used for karyotypeing of our index and her sons. Translocation(2,11) was detected in the index.We also examined her two sons for translocations and both of them were normal.There are two questions here: 1)Is this type of translaocation a strong cause for mental retardation of the neice and nephew? 2)If yes why the index case didn't have any sign of mental retardation? It seems that because our procedure cannot detect all the sub bands in a patient's chromosomes. Microdeletion or inversion may be change the phenotype in the cases nephew and neice. FISH or molecular methods can be help to find out the problem in this family.

P0401. Association of der(13;14) with primary amenorrhea

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Among 28000 karyotypes of peripheral blood performed in our center for various indications, 35 balanced translocations of chromosomes 13 and 14 have been detected. Of these 35, 26 were referred for history of abortions, IUFDs, offspring with congenital anomalies, and offspring with trisomy 13. Interestingly, 6 cases were 3 first cousin couples where both partners were carriers. The remaining 9 individuals were referred for reasons other than reporductive failure, 1 for mental retardation, another for premarital check up and the other 7 for primary amenorrhea.
Considering that 13 of the 35 translocation carriers were males, 7/23 der(13;14) carriers had primary amenorrhea. Cases referred for primary amenorrhea were aged 12-19 years without history of menstruation, and with one exception (12 years old) they had normal age related secondary sexual characteristic development, and growth.
Overall, 413/28000 cases were referred for primary amenorrhea who did not show related chromosomal changes such as 46,XY and 45,X variants. 10/363 had chromosomal aberrations including pericentric inversion of chromosome 1 and 2, paracentric inversion of chromosome 14 and the other 7 had der(13;14).
We would appreciate to be informed of similar findings and any guidance as to the etiology and possible association our data suggest.

P0402. Gonosomal Mosaicisms derived from a XY-Zygote

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Mosaicism is caused by postzygotic aberrant mitoses and leads to numerical or structural aberrations or a combination of both. The karyotype of the zygote can be normal or abnormal.
Our investigation group comprises 25 cases of gonosomal mosaicism with a Y-chromosome analysed in at least one cell system: 45,X/46,XY (n=8), 45,X/46,X,idic(Y)-mosaicism (n=7), 45,X/47,XYY-mosaicisms (n=5), 45,X/46,X,del(Y) (n=4) and 45,X/46,X,r(Y) (n=1). 13 patients were phenotypically male, 7 were female and 5 showed intersexual external genitalia.
The patients age at the time of chromosome investigation ranged from the prenatal period up to the age of 36 years.
The most frequent clinical findings were: growth retardation, abnormalities of the external genitalia, kidney malformations and different types of heart defect.
Cytogenetic investigations combined metaphase and interphase analyses with FISH (DNA probes: wcp X and Y; CEP X and Y; Yph 3.4). Between 2 and 5 cell systems per patient were analysed with regard to their origin from different blastodermic layers.
An unequal distribution of the mosaic could be demonstrated in the different cell systems, with no preferential combination.
Patients with dicentric Y-chromosome revealed the highest instability of karyotype. There was no age dependent karyotype changes in our investigation group.
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P0403. 12q22q24.33 Duplication: case report and review of the literature

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We present a case of partial duplication of the long arm of chromosome 12 characterized by FISH techniques using YAC probes.
On physical examination, at 8 years, our patient demonstrated: macrocephaly, flat occiput, long palpebral fissures, long eyelashes, protruding nasal root, anteverted nostrils, large and asymmetric ears, thin lips, light prognathism, malar hypoplasia, short stubby hands and femoral dysplasia. Magnetic resonance imaging showed partial agenesis of the cerebellar vermis and cystic dilatation of the fourth ventricle consistent with Dandy-Walker malformation.
A neurological and behavioural assessment revealed a psychomotor retardation and attention deficit/hyperactivity disorder (ADHD).
Standard cytogenetic analysis showed a partial duplication of the long arm of chromosome 12. Parents' karyotypes were normal.
To define the extension of the duplicated region we performed FISH analyses by using the following YAC probes (kindly supplied from YAC Screening Centre, Tigem, Milan, Italy): 943B11 (q21; 96.5 cM), 778F6 (q22; 97.6 cM), 850A12 (q22; 99.6 cM), 934C1 (q23; 108 cM), 827A10 (q24.1; 137.5 cM), 910B10 (q24; 154 cM), and 812D10

(q24.3; 161 cM). The analyses evidenced a tandem duplication of the 12q22q24.33 region with the proximal breakpoint located between 96.5 and 97.6 cM and the distal one between 154 and 161 cM resulting in the following karyotype: 46,XY,dup(12)(q22q24.33).
In order to contribute to the identification of the characteristic clinical features associated with 12q partial duplication we reviewed the literature and compared our case with those already described.

P0404. Ring chromosome 4 associated chromosome instability

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Ring chromosomes are rare abnormalities that typically arise de novo. The usual ring phenotype have been shown to have a wide range of intellectual functioning and congenital anomalies. We found several ring chromosome 4 instabilities in mosaic form with a normal cell line in peripheral blood cells of a 27 year old woman who was referred for infertility and short stature. The results of cytogenetic analysis showed 45,XX,-4/ 46,XX,r(4)/ 46,XX,dic r(4)/ 47,XX,r(4),+r(4)/ 46,XX karyotype in three repeated examinations. FISH analysis executed for precise characterization of the ring chromosome 4 breakpoints using chromosome 4p, 4q telomeric probes demonstrated deletion of the 4p telomere from the ring chromsome 4. Parental karyotypes were normal. After then, she became naturally pregnant and we performed amniocentesis at 16 weeks gestation. The fetal karyotype was normal. The phenotypes of our patient seemed to be related to the ring syndrome by the ring chromosome instability. The present study would be offer information to the long-term consequences of ring chromosome instability on clinical outcome.

P0405. A novel BAC probe set for the analysis of hematological malignancies

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Background: Translocations and deletions in the chromosome 11q23 region are frequent in hematologic neoplasms such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS) and chronic lymphocytic leukemia (CLL). We describe here the use of a bacterial artificial chromosome (BAC) probe set from the 11q23 region, applied to these malignancies.
Methods: Four BAC probes from one contig, and two BAC probes from another contig on 11q23 spanning the MLL region were labeled with either biotin or DIG for dual color fluorescence in situ hybridization (FISH) analysis. These probes were applied to cases with hematologic malignancies with known structural abnormalities involving 11q23.
Results: A split signal was detected in one ALL case with t(4;11)(q21;q23), one AML case with t(9;11)(p22;q23) and another AML case with t(11;19)(q23;p13), demonstrating that the probes span the MLL region known to be involved in these translocations.
The probes were further tested on one case of AML with add(11)(q23) and two cases of MDS and two cases of CLL, all with del(11)(q13q23). The additional material on 11q23 and marker chromosomes were found to be amplification of the regions detected by the probe set. Although the MDS and CLL cases cytogenetically appeared to have the same abnormality, the MDS cases had deletion of both contigs, while the CLL cases only had deletion of one contig.
Conclusions: At a molecular level, deletions in our CLL and MDS cases appeared different. This study demonstrates the importance of combining molecular and cytogenetic techniques to further define chromosome abnormalities.

P0406. Deletion of abl gene resulting from a recombination of a maternal (3;22;9)(q22;q12;q34.1) translocation in a child with axial hypotonia and dysmorphy.

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Small deletions near breakpoints may be an important cause of disease in apparently balanced chromosome rearrangements. We report on chromosomal findings in a boy with axial hypotonia and dysmorphy. He was born after a full-term uneventful pregnancy. He was the third child of healthy unrelated parents. The mother has had nine miscarriages. The child was first admitted at the age of 8 months for gastro-enteritis which was rapidly cured. Physical examination showed moderate axial hypotonia, hypertelorism and bilateral epicanthus, high-arched palate, macroglossia, and antimongoloid palpebral fissures. There was no evidence for visceral malformation. By conventional cytogenetic analysis a reciprocal balanced translocation between chromosomes 3 and 22 was diagnosed. In order to define clearly the breakpoints on the two chromosomes, chromosome painting was used and revealed a complex, apparently balanced translocation t(3;22;9). This translocation was inherited from the mother. Fluorescence in situ hybridization with different locus probes near breakpoints showed a deletion of abl gene located at 9q34.1 in the patient. This deletion was not found in the mother and in the sister carrying the translocation. Deletion of abl gene has been described once in a newborn with a complex cardiac anomaly and carrying a paracentric inversion of the long arm of chromosome 9 (Kleyman et al., Am J Med Genet, 1997).
Our case emphasises that molecular cytogenetic analysis of breakpoints in apparently balanced chromosomal translocations should be systematically carried out in patients with phenotypic abnormalities.

P0407. Prenatal Diagnosis of 45,X/47,XX,+8 mosaicism

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We report a case of prenatally diagnosed mosaicism 45,X/47,XX,+8. Prenatal diagnosis of mosaic trisomy 8 is relatively rare and the occurence of this abnormality in association with monosomy X is very unusual.
Our patient-24 years old primigravida -was reffered for abnormal ultrasound findings [agenesis corp.callosi,heart defect] at 33 t.g. Routine cytogenetic analysis of fetal blood [FB] cells and cultured amniotic fluid [AF] cells was performed. In FB cells the mosaic karyotype 45,X[10]/47,XX,+8[28] we found. All examined cultured AF cells were 45,X.
Results of cytogenetic analysis were compared with fluorescent in situ hybridization. FISH confirmed the mosaic karyotype 45,X/47,XX,+8 in FB cells and in uncultured amniocytes as well as in peripheral blood lymphocytes and buccal epithelial cells of the affected child after delivery. In cultured AF cells trisomy 8 was not found by FISH.
Our findings indicate the difficulties in the prenatal diagnosis of autosomal mosaicism, namely trisomy 8. This chromosomal abnormality can be missed with routine prenatal cytogenetic analysis because of a different tissue-specific distribution of the abnormal cell line.

P0408. The usage of FISH-WCP technique under the cytogenetical observation of 120 Chernobyl liquidators with different radiation doses

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FISH-WCP technique was introduced in Ukraine for the first time in cytogenetic lab of RCRM in 1999 in the frame of the Ukrainian-American project "Leukemia" for the comparison of different dosimetry methods (including biodosimetry) in Chernobyl liguidators. According to the protocol of the dosimetry part of the Project all cytogenetecal investigations had been fulfilled in blind manner. Method FISH (with directly labeled by Spectrum Orange DNA-probes to chromosomes 1, 2, 4) had been tested on the 120 liquidators of the Chernobyl accident ranging in age from 37 to 73 years in dose range from 10 till > 100 cGy. The data received confirmed that at present FISH-WCP technique can be successfully use in delayed terms following the radiation exposure for the indication of human irradiation and group dosimetry. As regard of the usage FISH method for individual retrospective dosimetry of radiation exposure especially in the range of low doses there are many problems of which needed in further investigations. At present the high and variable background

frequencies of complete translocations, strong age effect and essential interindividual variability in the rate of radiation-induced stable aberrations permit the use FISH for estimation and verification of doses in the part of Chernobyl liquidators with assumed radiation exposure more than 25 cGy.
This work had been supported by National Cancer Institute of USA in the frame of Ukrainian-American Project “Leukemia”.

P0409. Chromosomal analyses in infertile men.

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The incidence of chromosomal abnormalities in infertile males varied from 2.2 to 14.3%. The incidence of chromosomal variants was shown to be at 34.5% in infertile men and 10-15% in the general population. Our study was performed to determine the frequency of chromosomal abnormalities and polymorphism in infertile men with azoospermia and severe oligospermia (sperm density <5x10M/mL). Chromosomal analysis of 27 infertile men was performed both from peripheral blood lymphocytes and skin fibroblasts cultures using GTG, C banding, fluorescent in situ hybridization (FISH) and other methods. In 5 (18.5%) cases chromosomal abnormalities were found. There was one case (3.7%) with numerical chromosomal abnormality (47,XXY). Structural abnormalities were revealed in 4 cases (14.8%), from which 3 were mosaics (in 3%-98% of cells). The chromosomal variants were found in 8 (29.6%) patients. In 15 (55.6%) cases the karyotype was normal. We have found higher than given in the literature percentage of chromosomal abnormalities (mainly structural abnormalities), which could be reason of infertility. This finding could be explained partly by small amount of cases. We wish also to stress, that to reveal the low percentage mosaicism, it seems to be necessary to analyze at least 33-50 mitoses. However, the frequency of chromosomal variants in infertile men could support the opinion that chromosomal variants may be associated with reproductive failure in men.

P0410. A comparative analysis of G-banding, FISH and RT-PCR for the diagnosis of bcr-abl-positive CML

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Phildelphia (Ph) chromosome-positive CML, with the bcr-abl gene translocation, has a dismal prognosis. The identification of Ph-positive patients is vitally important because only aggressive therapeutic approaches, such as interferon alpha (IFN-alpha) treatment and allogeneic bone marrow transplantation, may result in long-term disease-free survival. Routine diagnostic methods for detection of the bcr-abl translocation include conventional cytogenetics(G-banding), reverse transcriptase-polymerase chain reaction (RT-PCR), and more recently, fluorescence in situ hybridization (FISH) analysis. Routine cytogenetics . sensitive at the initial diagnosis is unreliable during treatment. RT-PCR analysis is considered the most sensitive tool for the detection of the bcr-abl translocation, and is widely used alone, or in combination with the other methods. FISH analysis is simple, extremely reliable and sensitive. This study compares the efficiency of the three methods for the initial diagnosis of Ph-positive CML, and for detection of minimal residual disease during treatment. Conventional G-banding cytogenetics, FISH with BCR and ABL double-color probes and RT-PCR for detecting Ph-positive CML were undertaken in 22 CML patients undergoing either IFN-alpha treatment or allogeneic bone marrow transplantation (allo-BMT). The results obtained using the three methodological approaches were 100% correlated at diagnosis. Following treatment the cytogenetic analysis becomes technically less feasible and the results less reliable. Whereas, RT-PCR and FISH analysis remain equally and mutually contributive. We thus conclude that the concomitant use of FISH and RT-PCR remain the optimal diagnostic combination for the detection of the bcr-abl translocation.

P0411. Trisomy 12 mosaicism in a newborn presenting with chylothorax, facial dysmorphism, genital anomalies and congenital heart disease

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We present a newborn girl with trisomy 12 mosaicism,[46,XX/47,XX+21]. The girl was born at 39 weeks of gestation after an uneventful pregnancy to healthy parents.
After birth, chylothorax, congenital heart disease (VSD, ASD), muscle hypotonia, facial dysmorphism and ambiguous genitalia were cardinal features.
Chromosome analysis of various tissues revealed trisomy 12 mosaicism (skin 2/60 cells, muscle 3/43 cells, lung tissue 7/37 cells, blood and pleura: normal karyotype).
Complete trisomy 12 in humans is not viable. To our knowledge this is the tenth published case of trisomy 12 mosaicism in a liveborn. Obviously due to the nature of mosaicism, reported cases show a wide spectrum of anomalies ranging from Kartagener syndrome in an otherwise healthy man to multiple congenital abnormalities including complex heart malformations, facial dysmorphism, urogenital abnormalities (renal hypoplasia, ectopia vesicae) and further inner malformations (multiple accessory spleens, pancreatic-spleenic fusion, gallbladder hypoplasia) with neonatal death. No typical mosaic trisomy 12 phenotype can be delineated.
We report the first case of trisomy 12 mosaicism presenting with chylothorax, facial dysmorphism and genital anomalies as cardinal features. We compare our patient with earlier described cases.

P0412. Coincidental Structural And Numerical Aberrations Of The Chromosome 15 - Three Different Case Reports

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We report three cases of chromosome 15 aberrations. First one is a girl with dysmorphic features, seizures, and a delay of psychomotoric development. Her karyotype was 47,XX,+mar. Result of our FISH analysis was 47,XX,+mar.ish idic(15)(q11-q13)(D15Z1++,D15S10+,PML-). The second case is a 26-years old healthy pregnant woman, whose foetus has karyotype 47,XX,+mar. Our FISH result was 47,XX,+mar.ish dic(15)(D15Z1++,SNRPN-, PML-). The third case report desribes a 2-years old boy with blindness, deafness, dumbness and cryptoorchism.The cytogenetic analysis revealed a karyotype 45,XY,der(21), t(15;21)(q13;q22.3), -15. FISH confirmed the cytogenetic result: 45,XY,der(21), t(15;21)(q13;q22.3),-15.ish der(21)(D13Z1/D21Z1+,D15Z1-, SNRPN-, UBE3A/D15S10-, D21S270/D21S337/D21S55/D21S233+, PML+). The result indicates haploinsufficiency of the critical Prader-Willi/Angelman region in 15q11-q13. The work was supported by grant IGA NE5685-3 (Ministry of Health CR) and research project of the Charles University No.111300003.

P0413. Cytogenetic Analysis of 111 Cases of Adult Acute Lymphoblastic Leukaemia in an Asian Population.

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The majority of cases with acute lymphoblast leukemia (ALL) have abnormal chromosomes. Many of these abnormalities have been shown to be important clinical prognosticators. Cytogenetic analysis is therefore used routinely in management of ALL. In the current study we evaluate the cytogenetic abnormalities seen in a series of cases of adult ALL in an Asian population.
A total of 111 cases of ALL admitted to Singapore General Hospital between 1995 and 2001 were analysed. The age range was 16-81 (46 females, 65 males).
Chromosome preparations were made from bone marrow cells using direct and 24 hour cultures. Karyotypes were constructed in accordance with ISCN nomenclature.
Chromosome abnormalities were seen in 73% of cases a normal karyotype in 21.6% while the remaining 5.4% were unsuccessful. The most common balanced structural abnormalities were t(9;22)(q34;q11.2) seen in 24 (21.6%) and 1 variant translocation, followed by t(8;14)(q24;q32) in 5 (4.5%) with variant translocations in 4 additional cases. The diagnostically important abnormalities t(4;11)(q21;q23) were seen in 2 cases and der(19)t(1;19)(q23;p13) and it’s balanced form in 3 and 1 case respectively. Deletion of 9q was the most common region of deletion seen in 7 (6.3%) cases.

All the abnormal cases but 3 had structural abnormalities. A hypodiploid karyotype of <46 chromosomes was seen in 10.8 %, pseudodiploid in 41.4%, hyperdiploid of 47-50 in 12.6% and hyperdiploid >50 in 6.3% of cases respectively.
The most frequent numerical gains were for chromosomes 8, 21, 14, 18, while loss of chromosome 9 was the most common loss.

P0414. two new cases with 48, XXXX chromosome and review of the litterature.

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Although 47,XXX, 47,XXY, 47,XYY and 45,X karyotypes are frequent and occur at least 1 in 400 birth, patients with more than one extra sex chromosomes are very rare.
Here we report on two new cases with 48, XXXX (tetrasomy X) karyotype and reviewed the litterature. The pregnancy and delivery were normal in both cases. In case one, neonatal measurements were 2625 g for weight (- 1.5 SD), 46.5 cm for length (- 1.5 SD) and 34 cm for OFC (M). IUGR was noted in case two with 2100g for weight and 43 cm for length at birth. In both cases, facial dysmorphism including bilateral epicathus, synophris, long eyelashes, bulbous nose, long and flat filtrum, small mouth and bilateral radioulnar synostosis was noted. Evolution was marked by mild mental retardation with speech difficulties.
In attempt to well define the tetrasomy X, we reviewed the litterature. To our knowledge, since the first description of an abnormal number of chromosome in cultured lymphocytes reported by Lejeune et al in 1959, only 40 cases with 48,XXXX have been described in the litterature. We found that mental retardation was constant excluding one case with normal intelligence. Other features were particular facial dysmorphism, radioulnar synostosis and similar but non-specific behavioural problems. This syndrome seems to be clinically recognisable.

P0415. One event, two cell lines, three chromosomes, four breakpoints: unusual mosaic complex translocation in a patient with oligospermia.

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Complex chromosomal rearrangements and mosaic reciprocal translocations are rare events in constitutional cytogenetics. We report here on a 52 years old man with variable oligospermia who was referred to our laboratory for cytogenetic examination before AMP procedure. This man has two healthy children from a previous union. GTG and RHG banding revealed a complex translocation between chromosomes 9, 12 and 14 in 20% of the cells from two consecutive peripheral blood sampling. These standard banding techniques led to the proposal of the following chromosomal pattern: 46,XY,t(9;12;14)(q32;q13;q32)/46,XY.
Unexpectedly, FISH revealed a far more complex pattern, with two breakpoints on der(12). This derivative chromosome harbors material from chromosomes 9 and 14 hence correct designation is 46,XY,t(12;14;12;9)(q13;q32;p13;q32).ish t(12;14;12;9)(Tel14q+,wcp14+,wcp12+,wcp9+,Tel 9q+;wcp14+,wcp12+,Tel12q+;wcp9+,wcp12+, Tel 12p+)/46,XY. Careful hematological examination of the patient has been conducted to eliminate a rising malignant process.
During meiosis, these complex rearrangements are expected to form a multivalent from which multiple gametic combination can occur, most of which will be unbalanced. However, if female gametogenesis can accomodate itself to the complexity thrust upon it and produce balanced ovocytes, the rule of the greater vulnerability of spermatogenesis to structural rearrangement applies particularly in the case of complex abnormalities and heterozygote males are often sterile.
In our patient, the presence of a normal cell line may explain the more or less conserved fertility; however, in case of pregnancy following AMP, a prenatal diagnosis should be proposed to the parents as some of the imbalances might be viable.

P0416. From Mendelian inheritance to telomeres ...

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FISH studies using subtelomeric probes allowed us to explain MR/MCA recurrence in two large families with follow-up of 40 and 20 years respectively. Repeated high-resolution karyotypes were performed in both families. In the first family, a sex-linked recessive inheritance was suggested because two related male patients were observed. However a girl with the same clinical abnormalities was born recently. A multiprobe subtelomeric FISH study led to the identification of a familial cryptic translocation t(17;22)(q25;q13.3). A derivative 22 yielding monosomy 22q13 and trisomy 17q25 was observed in all affected patients tested. The translocation was segregated over at least four generations. In the second family, the observation of recurrent malformations and perinatal deaths over several generations suggested the presence of a familial rearrangement. Two cousins, a girl and a boy, with similar clinical findings died at a young age. Recently, photographs of both children were examined again. They showed facial dysmorphism evocative of a 2q27 deletion. FISH studies with chromosome 2 subtelomeric probes confirmed this imbalance and show that it derived from a cryptic familial t(2;17)(q37.3;q25). These observations illustrate that reassessment of " old " genetic files using both molecular cytogenetic tools and new syndromes clinical descriptions can point towards a correct diagnosis. In addition, they emphasise that recurrence of similar MR/MCA findings in different branches of a kinship is highly suggestive of a chromosomal anomaly. Genetic counselling and prenatal diagnosis are now available for both these large families.

P0417. Cat eye syndrome associated with a severe phenotype

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Cat Eye syndrome (CES) is characterized by a variety of congenital defects including ocular coloboma, anal atresia, preauricular tags or pits, heart and kidneys defects, dysmorphic facial features and mental retardation. The penetrance and severity are highly variable. This syndrome is associated with a supernumerary bisatellited dicentric marker arising from an inverted duplication of chromosome 22 classified into 2 types based on the location of the breakpoints. Most common CES chromosomes correspond to the smaller type I. We describe a female child with a severe phenotype of CES, born at 40 weeks of gestation from healthy parents. An intrauterine growth retardation was diagnosed at 22 weeks of gestation and was confirmed at birth. She developed a severe respiratory distress. She had hypotonia, dysmorphic features and malformations including downslanting palpebral fissures, hypertelorism, ocular coloboma, bilateral aplasia of the external ears, anal stenosis, patent ductus arteriosus. She died at age 1 month. Chromosome analysis showed a karyotype with an extranumerary bisatellited marker in all cells examined. The parents had normal karyotypes. FISH using 14/22 alpha satellite, WCP of chromosome 22 and N25 probes showed that the marker was a dicentric inverted duplication of the short arm and proximal long arm of chromosome 22, symmetrical and localized distally to the Digeorge locus (type II).
The severe phenotype of our patient demonstrated the phenotypic variability which does not seem to be related to the size of the duplication or presence of mosaïcism. This phenotypic variability increases the difficulty of genetic counselling.

P0418. Mapping of chromosomal breakpoints associated with orofacial clefts.

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As part of an EU-supported project aimed at the clinical, epidemiological and genetic study of cleft-lip-palate (EUROCRAN), we have initiated a search for candidate genes for orofacial clefts by mapping apparently balanced chromosomal breakpoints associated with cleft-lip-palate. The Mendelian Cytogenetics Network database (http://mcndb.imbg.ku.dk) contains information on 75 breakpoints in 25 individuals with oral clefting. Ordered YAC/BAC clones were used to map the breakpoints associated with two of these rearrangements: One involved a 46,XY,ins(9;5)(p22.2;q23.3q33.3) karyotype; the 5q33.3 breakpoint has been narrowed to a 250 kb region. Two overlapping BACs at the 9p22 breakpoint contain a hypothetical unknown gene, and the 5q23.3 breakpoint has been narrowed to a 150 kb region containing three candidate genes, including a putative zinc finger gene. None of these three breakpoints affects chromosomal regions previously known to harbor loci associated with orofacial clefts. The second case displayed an aberrant banding pattern on 7q36, a region previously implicated in congenital malformations affecting midline structures and a region included among those associated with orofacial clefts in more than one case in MCNdb (1p31, 4q21, 6p24, 7q36, 9p13, 16q24, 17q23 and 17p25). Chromosome 7 paint did not suggest the involvement of other chromosomes, the 7q-subtelomeric signals were normal, and high resolution CGH was normal, excluding deletions/duplications larger than ~3 Mb. This supports that the abnormal banding pattern is caused by a small intrachromosomal rearrangement, e.g. an inversion. We will try to solve this by a systematic FISH strategy using pooled BACs from 7q36.

P0419. Fetal isochromosome 18q: a case report and review of the literature.

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We report a female fetus with isochromosome 18q showing features of of both trisomy 18 and monosomy 18p. Few cases of this fetal syndrome have been published.
The pregnancy was uneventful, until prenatal sonographic examination at 20 weeks of gestation showed intra-uterine growth retardation (IUGR) and an encephalocele. The parents were non consanguineous, and had no relevant history of genetic diseases. Chromosomal examination of amniotic fluid cells showed an isochromosome 18q in all examined cells (46,XX,i(18q)). The fetus thus had trisomy 18q associated with monosomy 18p. The parents elected termination of pregnancy.
Fetopathologic examination revealed features of monosomy 18p (holoprosencephaly and facial malformations, occipital meningocele, heart and skeletal malformations), and trisomy 18q (microcephaly, short neck with pterygium colli, club feet, ovarian epithelial hypoplasia). In this case, the severity of the phenotype resulted in prenatal recognition of the chromosomal abnormality. Holoprosencephaly is not a common feature in post natal monosomy 18p (16% of all cases). It is thought to result from the deletion of HPE4, a gene encoding TGIF and located at 18q11.3.
Isochromosome 18q is thought to result from centromere fission (monocentric chromosome), or from chromatid exchange (dicentric chromosomes). Isochromosome (18q) is seen as a derivative chromosome; to our knowledge, no cases of supernumerary i(18q) have been reported, probably because tetrasomy 18q is not viable. The fetal features of isochromosome (18q) closely resembles the post natal phenotype, although the fetal syndrome may be more severe.

P0420. Isolated Agenesis of the Corpus Callosum and 8p Duplication

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We report a case of partial duplication of the short arm of the chromosome 8 associated with an isolated agenesis of the corpus callosum.
This is the first pregnancy of a young unrelated couple. During the second trimester an isolated agenesis of the corpus callosum was found.
A fetal blood and amniotic fluid sample were performed. The conventional karyotype showed an excess of material on the short arm of chromosome 8 suggesting a duplication. Parental karyotypes were normal. In situ hybridization with the whole chromosome painting of chromosome 8 confirmed the duplication. The couple chosed to interrupt the pregnancy. Anatomo-pathological examination showed a total agenesis of the corpus callosum associated with discrete dysmorphy. Array 300™ using the Genosensor™ System was used to confirm the duplication of 8p chromosome. Presently the Genosensor™ System includes 6 clones in the 8p region. 3 of them, the most centromeric ones, were abnormal detecting a duplication of the (8p11-8p22) region. We have to use BAC clones and FISH in order to precise the breakpoints of this duplicated fragment, and to try to reduce the critical zone of the corpus callosum on chromosome 8p.

P0421. Chromosome aberrraton frequency for medical staff exposed to ionizing radiation from open and close sources

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It has been evidenced that ionizing radiation induces microscopicaly detected changes in genetical structure of cell. Analysis of chromosome aberrations give informations about biological effects and biological responses of living organisms to ionizing radiation.
The aim of this paper was to show the results of chromosome aberration frequency analysis for persons occupationaly exposed to ionizing radiation from open and close sources.
We analised 106 health workers in period 1997-1999, and they were categorised into two groups according to the type of radiation they were exposed to: medical workers in nuclear medicine (24) who were exposed to ionizing radiation from open sources, and medical staff in diagnostic radiology (82) who were exposed to ionizing radiation from close sources.
The average duration of occupational exposition was 10.2 years for nuclear medicine staff, and 11.6 years for diagnostic radiology staff.
The results of cytogenetic examinations showed that chromosome aberration frequency was higher for nuclear medicine employers (in 4 persons-16.67%) than for the radiology employers (in 8 persons-8.54%), but there was no statistical significance (p=0.4622).
There was also no diference in chromosome aberration frequency for diferent professions in any of this group.

P0422. Testing the ability of the “Geno Sensor System” to measure the DNA copy numbers in 4 cell strains containing cytogenetically mapped abnormalities.

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Microarray bases genomic analysis is a novel technique designed for rapid detection of changes in numbers of human DNA specific sequence copies. The VYSIS GenoSensor™ System contains 287 human loci including single copy telomeric sequences, genes involved in micro-deletion syndromes, single copy sequences near the centromere and different loci reported to be amplified in various human cancers. We tested the ability of this system to measure single copy changes in 4 cell strains [a whole 21 chromosome gain (case 1), 2 deletions (1p deletion (case 2), 4p deletion (case 3)), one duplication 8p (case 4)].
The assay involves sample DNA labelling with Cy3 fluorophore. This is mixed with whole genomic reference DNA labelled with Cy5 fluorophore and co-hybridized to an Array 300™ microarray. After hybridization, target spots are analysed using the GenoSensor™ reader system. The proprietary software automatically identifies each spot and calculates for each target a normalized ratio that indicates the degree of gain or loss of copy number in the sample DNA.

All 21 clones were detected as gained for case 1. For cases 2 and 3 the detected DNA loss was in accordance with that found with other techniques results. For case 4 we confirmed the duplication of 8p, as well.
We now intend to use this system for the diagnosis of unknown changes in DNA copy number which might occur in different fetal or newborn pathologies with a probable genetic etiology where abnormalities have not yet been detected with other techniques.

P0423. Meiotic segregation analysis in three infertile patients with balanced reciprocal translocations

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Carriers of balanced reciprocal translocations may present with infertility, recurrent miscarriages, or offsprings with unbalanced karyotype and multiple congenital anomalies. If the risk of chromosomal imbalance may be estimated at birth, the implication of this imbalance for infertility and miscarriages is more difficult to determine. To assess the importance of these chromosomal anomalies during the gametogenesis, we have explored three infertile phenotypically normal men with reciprocal translocations. We performed meiotic segregation analysis of their sperm using FISH and PRINS methods. For patients 1 and 2, respectively with reciprocal translocations t(1;4)(q12;q28) and t(4;5)(q35;q34), more than 10000 spermatozoa were screened. In both cases, alternate segregation (giving phenotypically normal offspring) is the most frequent mode of segregation (68 and 70%) and the most frequent type of imbalance is adjacent 1 segregation (24 and 28%), corresponding to the most expected imbalance at birth. For patient 3 with reciprocal translocation t(1;21)(q11;q21) and with severe oligozoospermia, only 981 spermatozoa (from two ejaculates) could be screened. Alternate segregation is also the most frequent type of segregation (80%) and the two most frequent types of imbalance are adjacent 1 (10%) and 3:1 exchange (7,5%), this latter is responsible for the most expected imbalance at birth. Although alternate segregation is the major type of segregation, these three men have infertility. Likely there are other factors which can participate in the mechanisms of the reproductive failure, possibly from maternal origin as well. Meiotic segregation analysis in sperm is important to adjust a realistic management of the male infertility.

P0424. Detection of a partially cryptic complex chromosome translocation by FISH

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We report an “apparent” balanced *de novo* complex chromosome reorganization by the combined use of G-banding, FISH and CGH techniques in a two-year-old infant with some features vaguely reminiscent of trisomy 21. Conventional G-banding showed a complex chromosome translocation involving chromosomes 1, 4, 6 and 11. Multicolor FISH (24 colours) confirmed the presence of this complex chromosome translocation. A more complex reorganization was detected using whole chromosome painting probes for the chromosomes involved in this rearrangement. In this case we found two cryptic interstitial translocations in the same derivative chromosome: der(6) t(6;11;4;1;4). In the results obtained by CGH did not show any loss or gain of chromosome material in this patient. Our results indicate that the phenotypic abnormalities observed in this infant, with this “apparent” balanced complex rearrangement, may have resulted from either a small structural loss of material or a functional loss of a gene action.
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P 6. Diabetes

P0425. Polymorphism of *APOE* gene and its relationship to diabetic neuropathy in type 1 diabetes

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Neuropathy is a common complication of type 1 diabetes mellitus. These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum). A number of facts suggest that diabetic neuropathy may involve genetic susceptibility. Recently, it is reported that *APOE* polymorphism may influence to development of diabetic neuropathy. The gene encoding apolipoprotein E (*APOE*) has been proposed as a candidate gene for vascular complication in type 1 diabetes. Apolipoprotein E was discovered as a plasma protein involved in the metabolism of lipoproteins. There are three common alleles, *E2*, *E3*, and *E4*, which code for three major isoforms, resulting in six common genotypes. The aim of this study was to investigate the influence of *APOE* gene polymorphism in the development of diabetic neuropathy in type 1 diabetes patients. The study consists of 51 patients with diabetic neuropathy and 150 without diabetic neuropathy matched to the patients by age, gender and diabetes duration. *APOE* polymorphism was detected by the restriction fragment length polymorphism method after a polymerase chain reaction. The distribution of *APOE* genotypes and alleles frequency showed no difference between the patients with diabetic neuropathy and without this complication ($p>0.05$). The difference between the groups was tested by Fisher’s exact test. These results suggest that *APOE* gene polymorphism is no associated with diabetic neuropathy in type 1 diabetes patients.

P0426. A hypothesis about two kind of function of the CC-chemokine receptor CCR5 in the case of diabetes.

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We have investigated 38 diabetes Type I and 111 diabetes Type II Estonian patients. No statistical differences were found in CCR5delta32 allele frequencies in diabetes Type I and Type II patients (0,141 and 0,108, respectively)compared with the control group of native Estonian subjects (0,148). However, we have found that in diabetes Type I age of onset was 5,4 years later in patients carrying the CCR5delta32 mutation and they have less late complications ($p<0,03$). In diabetes Type II there was a difference in the frequency of concomitant diseases ($p=0,0006$), including obesity, thyroid diseases, neoplasias ect., being higher in CCR5 wild-type genotype. From these data we can draw a conclusion, that reduced CCR5 concentration may be useful in hyperglycemic conditions. We propose, that CCR5 has two types of functions: the well-known is to bind chemokines and activate of immune cells, and the second is, as we propose, regulation of functional state of cell membrane, in some conditions also cell contacts by activating Ras and Rho proteins of target-cells. Chemokine binding selectivity is mediated by the second extracellular loop, while affinity-sensitive binding is dependent on the NH2-terminus and the first extracellular loop of CCR5.In the hyperglycemic conditions an unspecific glycosylation takes place, which changes the affinity of ligand binding. It could make the ligand-receptor complex more persistent and extend the time of Gi receptor activated state. During chronic hyperglycemic conditions the altered functional state of cell membrane might lead to different expression of late complications and/or concomitant diseases depending on the CCR5 genotype.

P0427. Analysis of triplet repeat polymorphism in the transmembrane region of the MHC class I chain-related A (MICA) gene in siblings of diabetes with high ICA level.

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Insulin-dependent diabetes mellitus 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 ß cell, which leads to severe insulin deficiency. Recent studies have been demonstrated that polymorphism of MICA gene (major histocompatibility complex class 1 chain-related genes) are associated with supceptibility to type 1 diabetes. A total of 34 initially unaffected siblings with the level of cytoplasmatic islet cell antibodies (ICA) titer above 28 Juvenile Diabetes Foundation Units (JDFU) and 42 unrelated non-diabetic peoples from North-West region of Russia were analyzed for exon 5 polymorphism of MICA gene by DNA heteroduplex analysis. MICA gene has a triplet repeat polymorphism in the transmembrane region consisting of five alleles. The frequencies alleles 4, 5, 5.1, 6, 9 were 13.3%, 8.3%, 43.3%, 16.7%, 18.3% in study group and 8.3%, 16.7%, 31%, 17.8%, 26.2% in control group accordingly. More then 80% siblings were homozygote or heterozygote for A5.1 allele compared to only 57% of these subject in the control group (c2= 4,11, p<0,05).

P0428. Gene polymorphisms of the angiotensin I-converting enzyme and angiotensin II type 1 receptor A1166C in non-insulin dependent diabetes mellitus

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Background: Patients with non-insulin dependent diabetes mellitus (NIDDM) have high risk of developing micro and macro vascular complications and hypertension. ACE gene polymorphism is associated with an increased risk of vascular disease and AT1 gene polymorphism with hypertension. The aim of this case/control study was to determine genotypes for gene polymorphisms of the angiotensin I-converting enzyme (ACE-ID) and angiotensin II type 1 receptor (AT1-A1166C) in NIDDM patients and control group. **Methods**: We examined 40 patients (preliminary study) with NIDDM and 100 healthy controls. Genomic DNA was amplified by PCR method for I/D and A1166C polymorphism. **Results**: Frequencies for II, ID, DD ACE genotypes in patients were 16,67%, 59, 52%, 23,81% respectively and I/D allele frequencies were 0,46/0,54, not significantly different from our healthy population. Frequencies for AT1 genotypes in patients were 39,0%, 43,9%, 17,1% for AA, AC, CC, respectively. Significant difference between patients and controls was found for genotype distribution for A1166C polymorphism (p<0,05). Allele C ^{1166} was slightly but not significantly frequent in patients than in controls (0,39 vs. 0,27). **Conclusion**: Further study with larger number of patients with micro and macrovascular complications could reveal possible association of ACE and AT1 genes polymorphism with progression of these complications.

P0429. Design of the Family Investigation of Nephropathy and Diabetes (FIND)

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FIND is a multi-center consortium acquiring DNA from sets of families or individuals with well-characterized diabetic nephropathy and performing a genome scan to identify chromosomal regions linked with diabetic nephropathy. FIND includes families of African-, European-, Native- and Hispanic-American ethnicity. Affected sibling pair, discordant sibling pair, affected relative pair, and discordant relative pair linkage analyses will be performed in the “family” arm of the study. Mapping by Admixture Linkage Disequilibrium analyses will be performed in the “MALD” arm. Markers at candidate genes or chromosomal regions containing putative renal failure susceptibility genes identified in previous genome scans, or syntenic to regions of renal failure susceptibility in model organisms, will be examined. Approximately 10,000 individuals will be recruited in FIND. Participants provide medical histories and urine, plasma, and serum samples for determination of glycosylated hemoglobin, serum and urine creatinine concentration, and urinary albumin:creatinine ratio (UAC). Lymphocytes from eligible participants are immortalized and a repository kept for serum, plasma and urine samples. The family study is recruiting multiplex diabetic families identified by a proband with overt nephropathy or end-stage renal disease (ESRD) attributed to diabetes mellitus. Family eligibility is determined by at least one additional diabetic sibling with either nephropathy (UAC > 0.03 mg/mg) or without nephropathy (UAC < 0.03 mg/mg and normal serum creatinine concentration) after ten years’ diabetes duration. FIND will contain a large collection of multiplex diabetic families enriched for the presence of nephropathy. These resources should provide significant power to detect genes contributing to diabetic kidney disease.

P0430. Heritability of blood pressure phenotypes in type 2 diabetes

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High blood pressure is a recognized cardiovascular risk factor. Although emphasis in treatment has focused on lowering diastolic blood pressure (DBP) and pulse pressure (PP), PP may be a better predictor of events. The role of genetic factors in blood pressure, including systolic (SBP) and DBP, is well known. Fewer data are available on the extent of genetic control of PP. We hypothesize that in families with multiple members having diabetes, blood pressure phenotypes are influenced by both genetic and environmental factors. To determine the extent of the familial aggregation of SBP, DBP and PP, we studied 245 individuals with type 2 diabetes from 122 families. Other measured factors included duration of diabetes, ethnicity, body mass index (BMI), cigarette smoking, and self-reported medical history. Heritability estimates were obtained using genetic variance components (SOLAR). The sample was 89% Caucasian, 59% female and had a mean±SD for age and duration of diabetes of 60.6±10.4 and 11.2±7.9 years, respectively. Adjusting for age, gender and ethnicity, heritability (h^2±SE) for SBP, DBP and PP was estimated to be 0.21±0.18 (P=0.13), 0.35±0.16 (P=0.01), and 0.38±0.17 (P=0.01). No changes in residual heritability were noted after further adjusting for BMI, duration of diabetes or current smoking status. Adjustment for diagnosis of hypertension only slightly altered the residual heritability estimates (0.26 for SBP, 0.33 for DBP, 0.37 for PP). These data provide empirical evidence that blood pressure phenotypes, including pulse pressure, have a significant genetic component in families with multiple members with type 2 diabetes.

P0431. AIRE Gene Polymorphisms in Finnish Patients with Type 1 Diabetes

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Type 1 diabetes (IDDM) is an autoimmune multifactorial disease characterized by destruction of the pancreatic islet cells, with the major susceptibility gene in HLA region on chromosome 6p21. IDDM often occurs in autoimmune polyendocrinopathy syndromes (APS). In APS1, a rare autosomal recessive autoimmune disease with Mendelian inheritance, the patients have IDDM independently of the HLA association. Nevertheless, IDDM in APS1 patients strongly resembles HLA-associated IDDM with high titers of GAD65 autoantibodies and specific lymphocytic destruction of pancreatic ß-cells. The gene responsible for APS1 - AIRE (autoimmune regulator) - has been mapped to chromosome 21q22.3 and characterised as a transcriptional activator. IDDM and APS1 are enriched in Finnish population. We designed a DNA chip with AIRE sequence polymorphisms and mutations searching for allelic variants associated with IDDM. For analysis we used arrayed primer extension (APEX) technology, a resequencing method based on two-dimensional array of oligonucleotides. We report the allelic and genotype frequencies for 11 SNPs in 123 patients with IDDM and 135 controls from the Finnish population.
The genotype frequency of two SNP-s, 1197T>C and 1411C>T was increased among patients, compared to controls. We suggest that 1411C>T polymorphism, resulting in amino acid change R471C, may be associated with IDDM autoimmunity in some of the Finnish patients. We also show that the most common APS1 mutations, R257X and 13-bp deletion in exon 8, as well as the third Finnish mutation K83E, do not contribute to a haplotype carrying susceptibility to IDDM.

P 7. Gene Structure and Function

P0432. Microsatellite repeat analysis of Xq27 region in Belarus population

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Flanking microsatellite markers and their association with instability of the FMR-1 CGG repeat, involved in the fragile X syndrome, were analyzed in DNA samples from Belarus population. Comparison of DXS548-FRAXAC1-FRAXAC2 haplotype frequencies in the 189 normal, 22 intermediate and 9 fragile X chromosomes suggested a strong linkage disequilibrium between normal alleles and haplotype 7-3-4+ as well as between expanded alleles and haplotype 2-1-3. Haplotype 2-1-3 was reported to be significantly enriched among high-end normal alleles. In our study we also revealed a number of closely-allied haplotypes (1-1-3, 2-1-2, 3-1-3) which are almost exclusively linked with “gray zone” CGG repeats. We suggest that this group could arise from the founder chromosome with haplotype 2-1-3 and intermediate size FRAXA allele, by the series of single mutational events, by mechanism similar to replication slippage. It means that such chromosome must have a higher rate of replication mistakes in this region or inability of repair system to correct changes naturally occurring in repetitive sequences. Remarkably, that the same mechanism was proposed for gradual progression of intermediate alleles towards instability threshold. Instability in both FMR-1 and the flanking markers in Xq27 can be influenced by a common trans-acting factor promoting general instability in microsatellite sequences. It can also explain the existence of the haplotypes which are rare or absent in the control samples but are well represented in intermediate and fragile X chromosomes.

P0433. Possible involvement of chi-like and minisatellite sequences in the formation of two chimeric CYP21P/CYP21 genes in steroid 21-hydroxylase deficiency

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Congenital adrenal hyperplasia (CAH) is a common autosomal recessive disorder mainly caused by defects in the steroid 21-hydroxylase (CYP21) gene. More than 90% of CAH cases are caused by mutations of the CYP21 gene. Approximately 75% of the defective CYP21 genes are generated through intergenic recombination termed “apparent gene conversion” from the neighboring CYP21P pseudogene. A chimeric CYP21P/CYP21 gene with its 5’ end corresponding to CYP21P and 3’ end corresponding to CYP21 has been identified. This kind of gene is nonfunctional because it produces a truncated protein. We have found that there are two distinct chimeric genes in CAH patients. Both had a sequence with -300 nucleotides of the 5’ head as the CYP21P gene. Otherwise, the coding region consisted of a fusion molecule with the CYP21P gene in two different regions. One of the junctions was located in chi-like sequence of GCTGGGC in the third intron and the other was in the minisatillite consensus TGGCAGGAGG of the exon 5 of the CYP21P gene. In addition, the analysis of restriction fragments length polymorphism for these two 3.3-kb chimeric molecules showed these sequences arose as a consequence of unequal crossover between the CYP21P and CYP21 genes. It is plausible that both consensus sequences may be responsible for the gene conversion of these two chimeric genes.

P0434. Sertoli cell-germ cell interaction: Functional analysis of murine calgizzarin gene.

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In the present study we undertook functional analysis of a differentially regulated gene, named calgizzarin, which shows a decreased expression in murine Sertoli cell-germ cell cocultures compared to cultured Sertoli cells alone. Calgizzarin is expressed in low level in all adult tissues examined; however, a high mRNA level for calgizzarin in mouse testis is maintained until stage P15 and then declines dramatically, whereas the expression pattern in the ovary remains constantly high throughout development. Therefore its expression in Sertoli cells might be repressed by some unknown factor/s originated from spermatocytes/spermatids. The gene is approximately 6 kb pairs in size and contains three exons. The ORF encodes for a 98 amino acid polypeptide containing two putative EF-hand calcium-binding domains. To elucidate the potential role of calgizzarin gene, we decided to delete the gene in mouse by targeted disruption (knock-out). A replacement-targeting vector was designed to delete the two exons encoding calgizzarin protein and replaced them with the neomycin phosphotransferase gene. The ES cell were transfected with the targeting vector and selected for homologous recombination event. Five recombinant clones were identified by genomic Southern. Two clones produced germ line transmitting chimeras after injecting them into blastocyst derived from C57B/J6 female. These chimeras were bred with C57B/J6 female to generate F1 animals, which were heterozygous for calgizzarin deleted allele. Generation of homozygous animal is awaited. The phenotypic and molecular analysis of these mice might give us insights, about the potential role of calgizzarin in spermatogenesis.

P0435. Novel mutations in the RFXANK gene. Promotor binding of mutant RFX without MHC II transactivation.

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MHC class II deficiency or bare lymphocyte syndrome is a combined immunodeficiency caused by defects in MHC specific regulatory factors that control MHC II expression at transcriptional level. MHC II expression is controlled at least by four trans-acting genes: CIITA, RFXANK, RFX5 and RFXAP. RFXANK encodes a subunit of the tripartide RFX transcription complex that functions in the assembly of multiple transcription factors on MHC II promoters. It has four ankyrin repeats important for interaction with RFXAP and CIITA. So far seven different RFXANK mutations have been reported in 26 unrelated patients. The most frequent mutation - deletion of 26 bp (752delG-25) was identified in 17 patients. The other mutations are nonsense or splice site mutations leading to proteins lacking all or part of the ankyrin repeat region. Here we report two novel point mutations of RFXANK gene: D121V and R212X found in two families with BLS and additional studies on tyrosine residues 224Y and 235 Y located in fourth ankyrin domain. We found D121V allele is expressed in vitro but is unable to form, with other factors, stable RFX complex. The experimental mutant Y224A is able to form RFX complex which binds to the MHC II promoter in vitro but cannot reverse BLS phenotype. These data the importance of the D121 and Y224 residues deduced from modelling studies.

P0436. Functional and genomic analysis of the SIX gene family

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Members of the SIX gene family are involved in a variety of developmental processes in vertebrates and invertebrates. All SIX genes encode two highly conserved motifs, a homeodomain that mediates DNA binding, and a SIX domain that mediates protein-protein interactions. It is now clear that Drosophila has three SIX genes (sine oculis, Optix and D-Six4) while humans have six (SIX1-6). Phylogenetic analysis of Drosophila and mammalian amino acid sequences shows that the proteins fall into three clear subgroups, suggesting that a common ancestral organism possessed three SIX genes. An intriguing feature of the human SIX genes is their genomic organisation, as revealed by the Human Genome Sequencing Project; five of the six genes are clustered. We have shown that the mouse SIX genes appear to have a similar genomic arrangement. Despite extensive amino acid homology within the SIX-domain, SIX proteins differ dramatically in their interactions with transcriptional co-factors encoded by the EYA gene family. We report work in progress which shows that while SIX1 interacts strongly with EYA2 and EYA4 in a yeast two-hybrid assay, SIX3 interacts with neither. This implies that there are clear functional differences between the subgroups of SIX proteins.

P0437. Genetic background of the protein regulation of FGF2 ex vivo

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The potent cytokine FGF2 is involved in the proliferative response to vascular injury of many cell types and is therefore suggested to be an important candidate gene for CAD. **Materials and methods:** We investigated the individual FGF2 protein- (ELISA; R&D-System) and mRNA-expression (competitive RT-PCR) in 99 patients with CAD (49.9y, SD 8.7, 85 males). Moreover we examined the potential role of the exon1-polymorphism C223T of FGF2 in regulating the mRNA- and protein-expression pattern. This polymorphism, located in the 5'-UTR in the functionally important ribosome entry site, modifies the calculated mRNA folding-structure and may therefore influence the expression of FGF2. **Results:** Investigating the FGF2 expression on transcriptional and translational level we determined an mRNA expression of 13.23 ag/cell and a protein expression of 49.19 pg/ml serum in our patient group. Furthermore a significant positive correlation of FGF2 mRNA and protein expression could be detected ($p<0.001$). The study of the genotype distribution of the C223T-polymorphism resulted in the following frequencies: 0.84 (CC); 0.14 (CT); 0.02 (TT). An analysis of the influence of the C223T-polymorphism on the FGF2 expression revealed significant correlations on transcriptional ($p<0.01$) as well as on translational level ($p<0.001$). The highest values of both mRNA- and protein-expression were determined among CC-carriers (14.3ag/cell; 54.4pg/ml serum), whereas CT-carriers showed intermediated values (8.3ag/cell; 23.2pg/ml serum). The lowest values were found among TT-carriers: 3.3ag/cell; 14.5pg/ml serum. **Conclusions:** The FGF2 mRNA- and protein-expression was shown to be significant dependent on the C223T-polymorphism. Further investigations regarding the FGF2 expression pattern should bear this association in mind.

P0438. Promoter and mRNA analysis indicate low expression level of SLC7A7 gene.

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The SLC7A7 gene encodes a cationic amino acid transporter y+LAT1 expressed mainly in basolateral compartment of epithelial cells in intestine and renal tubules. Mutations of SLC7A7 cause a rare but severe aminoaciduria, lysinuric protein intolerance (LPI). We have investigated putative promoter regions of SLC7A7 using luciferase reporter gene and electrophoretic mobility shift assays (EMSA). The expression level of the gene in LPI patients and controls was also studied.
The 5' region of the first untranslated exon of SLC7A7 contains no classical promoter elements like TATA-box or Sp1 binding sites. However, it includes several other putative transcription factor binding sequences, like CACATG and CCCCTGGC, sufficient to promote luciferase expression in human embryonic kidney cells (HEK-293). The rise of the luciferase activity was 10-fold in comparison with the negative control; no activity was observed in fibroblasts. Accordingly, by EMSA we demonstrated that the sequence elements were able to bind protein in HEK-293 cells and adult kidney tissue extract, but not in fibroblasts. Northern blot analysis showed very low and equal SLC7A7 mRNA levels in control, carrier and LPI patient fibroblasts. In conclusion, it is likely that identified transcription factor binding sites contribute to the low expression level and the epithelial specific regulation of the SLC7A7 gene.

P0439. Experimental verification of predicted splice variants of human genes

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Public EST databases currently contain more than 3 million human EST sequences, representing probably 40-50.000 human genes. Within these data exists a large redundancy. We take advantage of this redundancy by analysing the differences of sequences belonging to the same gene. The EST sequences are clustered and assembled to a consensus sequence. However, many clusters cannot be assembled into a single consensus sequence. The sequences then fall into multiple consensus sequences (contigs) within one clusters. The differences might be due to imperfect sequence data (e.g. partially unspliced sequence templates) or due to alternative splicing. Instead of one gene coding for one mRNA leading to one protein, alternative splicing of transcripts may lead to potentially different proteins.
Splice variants are often due to alternative exon usage, which we verify by RT-PCR.
We have set up a medium throughput strategy that does allow us to screen expression of genes in 31 different human tissues. We initiated this project by analysing genes on Chromosome 21 and 22. Our results indicate, that the theoretical data represented in EST databases can be verified in many cases by our experimental design. We re-sequence PCR products in question, to confirm their origin and nature. In more than 35% of the cases, we cannot experimentally support EST data by RT-PCR.
In future we want to extend splice variant analysis to other chromosomes and gene families. We intend to automate RT-PCR and ultimately design a chip to discriminate a large number of different splice forms of medically relevant genes.

P0440. RET upstream sequences modulate gene expression via cell-line specific chromatin acetylation.

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Disregulation of the RET proto-oncogene transcription may play a role in both inherited cancer syndromes and Hirschsprung disease. Understanding the gene regulation might provide new clues to clarify pathogenesis. Recently, we reported that RET transcription is highly cell-line specific, while the promoter region is equally active in different cell-lines.
Here we show that RET upstream sequences can modulate gene expression via cell-line specific chromatin acetylation level contributing to promoter function. Acetylation and deacetylation activities, working on specific lysines of histonic tails, alter the accessibility of transcription factors to DNA thus modulating gene expression. Histone deacetylase inhibitors, such as sodium butyrate, up regulate both RET mRNA levels and transcription rate in a RET expressing cell-line. The same treatment allows transcript detection in RET negative cell lines (lymphoblasts), while no enhancement is seen in cells expressing RET already at high level. Sensitivity to sodium butyrate appears to be sequence specific in transient transfection assays. Chromatin immunoprecipitation experiments, using antibody against H4 tetra-acetylated hystones, confirm the direct role of histone acetylation level within RET upstream region, in regulating gene expression.
Sodium butyrate derepressive effect allowed us to overcome the lack of this gene expression in the majority of adult cells and analyse RET mRNA from Hirschsprung patients, by treating lymphoblastoid cell lines. Anomalous transcripts, defective gene expression, as well as nucleotide substitutions in the coding region were detected.

P0441. Genomic organization of human complexin 2 gene

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We have investigated the structure of the genomic clone Ghfb hybridizing with the brainspecific cDNA clone Hfb1 and obtained from the cosmid chromosome V library. We have shown Hfb1 to be a fragment of the 3'-terminal exon of the human synaptic protein complexin 2. This exon encodes the extended 3'-untranslated mRNA region. Sequencing of the genomic clone Ghfb let us determine the complete structure of three exons (3'-terminal and two neighboring) and two corresponding introns of the complexin 2 gene. The nucleotide sequence of three named exons includes the partial 5'- untranslated region, the ORF and 3'-untranslated region of the corresponding mRNA. Nucleotide sequence of the clone from the human genomic sequences database (AC010241) showing the highest similarity to Ghfb allowed us to determine the sequences of two another exons and two corresponding introns. These exons

represent the 5'-end of the 5'-untranslated region of the complexin 2 mRNA. Computer analysis of the human EST database proposed the existence of an additional exon within the intron 2 sequence at the distance of about 7 kb from the exon III. The resulting alternative transcript is thought to differ with its 5'-end from that for the previously described transcript. Using RT-PCR technique we confirmed the existence of the predicted transcript.

P0442. The comparison of substrate recognizing regions of Cytochrome P450 2C subfamily enzymes suggests the diversity-enhancing evolution of these regions during the evolution.

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The enzymes belonging to cytochrome P450 2C subfamily metabolize numerous drugs in liver. Four enzymes (2C8, 2C9, 2C18 and 2C19) members of this subfamily have been identified in humans. These enzymes play roles in metabolizing various toxic compounds. Considering the diverse nature of the chemical structure of these compounds, it is reasonable to assume that the substrate recognizing regions of these enzymes undergo diversity-enhancing evolution just as the antigen-binding regions of major histocompatibility complex. The test for the diversity-enhancing evolution is performed by the comparison of synonymous (silent) nucleotide changes versus nonsynonymous (amino acid altering) nucleotide changes during the molecular evolution. If nonsynonymous substitution is estimated to have occurred more frequently than synonymous substitution, then we suspect that diversity-enhancing evolution might have occurred. Cytochrome P450 2C subfamily has 6 substrate recognizing regions. By the analysis using a window with 30 codons, nonsynonymous substitutions were found to augment near the substrate recognizing regions. When the comparison was made between synonymous and nonsynonymous changes in cytochrome P450 2C subfamily enzymes, the diversity-enhancing substitutions were observed in different substrate recognizing regions. The hypothesis of diversity-enhancing evolution was tested by the simulation experiments and it was supported in almost cases with significance. Similar results were obtained in rabbit, rat and golden hamster. These results suggest that the substrate recognizing regions of cytochrome P450 2C subfamily enzymes have undergone diversity-enhancing evolution due to the selection pressure favoring the diversity adapting to the environment in which a variety of possibly toxic compounds attack the species.

P0443. Zinc fingers on short arm of chromosome 9 pointing to lymphocytes

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Genes encoding for C2H2 zinc finger proteins are known to regulate normal cell proliferation and differentiation and are often involved in tumor growth regulation. Therefore we chose this protein motif consensus as a virtual probe for in silico analysis of EST databases and genomic sequences from 9p22, a chromosomal region of our interest. Assembling physically clustered positive sequence fragments we detected some putative exons of an uncharacterised gene, which were then used for primer design to screen transcript pools from 12 tissues and 8 cell lines generated by RT-PCR. The corresponding full-length cDNAs from human peripheral blood leukocytes were sequenced and the exon-intron structure of the gene was determined. Two main transcripts of the novel gene were visualized by Northern blot hybridization.
The human gene designated as lymphocyclin contains 8 exons spanning approximately 450kb of genomic DNA in 9p22.2-22.3 between markers D9S156(tel) and D9S1218(cen). It has two main transcripts of 3.82kb and 0.84kb and also a splice variant lacking exon 2 (126nt). The longest transcript encodes a 1099aa protein with three pairs of adjacent C2H2 zinc fingers close to its C-terminus and has three putative nuclear localization signals. Other significant features of lymphocyclin are a 22aa leucine zipper motif and an alpha-helix forming a serine stripe.
Our experimental data so far indicate quite restricted expression of this new member of C2H2 zinc finger protein gene superfamily and refer to its possible role as lymphocyte differentiation and/or proliferation regulator.

P0444. FOXL2: Evolution and expression.

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Mutations in the FOXL2 gene have recently been shown to cause the blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), a rare genetic disorder. There are two types of BPES. In BPES type I, a complex eyelid malformation is associated with premature ovarian failure (POF) while in BPES type II, the eyelid malformation occurs as an isolated entity. The FOXL2 protein belongs to the family of forkhead transcription factors. These proteins have a highly conserved 100 amino-acid DNA-binding domain (the forkhead domain) and are involved in different developmental processes. The FOXL2 protein also contains a polyalanine tract that might have a role in transcriptional repression.
In order to study the FOXL2 locus (ORF and promoter), we have performed a comparative analysis of the sequence in several species including human, goat and mouse. The amino-acid sequences are highly similar and conservation is further demonstrated by low Ka/Ks ratios. From the alignment of the 5'upstream sequences in these species, we have deduced the sequence of a "core" promoter.
In addition, we have produced and characterized two polyclonal antibodies directed against the N- and C-terminal regions of the human FOXL2 protein which are also capable of recognizing the other orthologs. Immunohistochemistry shows that FOXL2 is a very early marker of ovarian development as it is expressed in follicular cells. The domain of expression of FOXL2 seems to restrict as development proceeds in the goat ovary.

P0445. EBV infected LCL human cells exhibit high expression of p53 gene

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Infection of B-lymphocytes with Epstein-Barr Virus in vitro induces a G0 to G1 transition followed by DNA synthesis and cell division. Infected cells undergo blast transformation resulting in the outgrowth of immortal lymphoblastoid cell lines. Numerous cellular proteins are switched on in the infected cells, including p53. DNA viruses encode oncoproteins that modulate the level of p53 and stimulate host cell DNA synthesis. It was reported earlier that p53 can be bounded by EBV gene products: EBNA5 nad BZLF-1 leading to its accumulations. We have investigated expression of p53 and bcl-2 genes on the level of its mRNA and protein in LCL human cell lines established from the blasts of EBV infected patients. For estimations of mRNA we have used RN-ase Protection Assay (h-CC2 multiprobe template set), the protein level was studied immunocytochemically. In our study we have observed increased level of p53 mRNA and protein expression, similary to observed in mitogene stimulated peripherial blood lymphocytes from healthy donors.
The increased level of p53 and bcl-2 expression in EBV infected LCL may suggest that transcription of p53 might be activated by EBV infection. As long as it is physiologically tolerable level it doesn't lead to growth arrest or apoptosis but promote proliferation.

P0446. Characterization, expression pattern, and chromosomal assignment of the novel human RAB22A gene belonging to Rab small GTPases

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The mouse chromosome 2 segment (MMU2) corresponding to human chromosome 20 (HSA20) is known to be involved in both, maternal as well as paternal noncomplementation (genomic imprinting). Uniparental disomies for distinct regions of MMU2 result in different neonatal lethalities with opposite anomalous phenotypes, strongly suggesting the presence of imprinted genes in this region. These chromosomal regions show a conserved synteny

of gene loci to human 20q13 segment, predicting the presence of imprinted genes in this syntenic human chromosomal region. We have identified a new gene in this region of interest which is located on a BAC RPCIB753L051096 proximal to GNAS1 on HSA 20q13. Sequencing the full-length cDNA revealed a novel isoform of the human RAB22 subfamily of small GTP-binding proteins which are located in distinct intracellular compartments and plays an important role in the regulation of vesicular trafficking. Based on the EST WI-12997 this new isoform was isolated containing 2242 nucleotides and is designated RAB22A. Structurally, the RAB22A encodes a polypeptide of 194 amino acids which has 97% identity to the canine rab22. Northern blot analysis revealed ubiquitous expression slightly increased in heart. The genomic structure was completed by database analysis and sequencing of the isolated BAC clone RPCIB753L051096. The gene consists of 7 exons spanning about 50 kb of genomic sequence. Physical and FISH mapping revealed that RAB22A is located proximal to GNAS1 but downstream of PCK1 on human chromosome 20q13. Supported by DHGP.

P0447. Phylogeny of the alternative NF1 exon 10a-2 reveals that intron sequences surrounding alternatively spliced exons are highly conserved

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We recently found an additional splice product of the Neurofibromatosis type 1 gene (NF1) with the new 45 bp exon 10a-2 inserted between exon 10a and 10b. Phylogenetic analysis of gDNA revealed the origin as well as the demise of exon 10a-2 in evolution. 10a-2 homologous sequences are not present in Drosophila melanogaster but in Fugu rubripes. It is expressed and highly conserved in birds and mammals but independently lost again in mouse, rat, sheep, cow and dog. Intronic sequences surrounding exon 10a-2 are highly conserved over more than 600 bp among mammals expressing 10a-2. We therefore suggest a specific function of exon 10a-2 and the surrounding intronic sequences lost again in some mammalian species. Extended intron homology, uncommon for NF1, was also found around three other alternatively spliced NF1 exons, one of which is rodent-specific (23b). Investigation of two other genes, the cystic fibrosis transmembrane conductance regulator (CFTR) and the Wilms tumor 1 (WT1) gene, also revealed high intron sequence conservation around the alternative CFTR exon 10b and WT1 exons 5 and 9. We therefore speculate that high conservation of the intronic sequences surrounding exons is related to alternative splicing.

P0448. Differential HFE gene expression in Caco-2 intestinal cells

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The human HFE gene is clearly involved in hereditary hemochromatosis a common genetic disorder in caucasian populations which is characterized by duodenal iron hyperabsorption. However, the precise function of the HFE protein and its role in the pathogenesis of the disease are still unknown. A possible role in homeostatic iron regulation is suggested by the co-localization and the interaction of the HFE protein with the transferrin receptor. We recently examined the functional organization of the HFE gene promoter that did not revealed any iron responsive element. Here, we studied the HFE gene expression in Caco-2 cells along with differentiation and holo-transferrin treatment. We used Caco-2 cells that differentiate with morphological and biochemical features of mature intestine enterocytes as a model to study the HFE mRNA expression. We examined the HFE mRNA level using real-time PCR in Caco-2 human intestinal cells grown on polycarbonate membrane inserts under different transferrin-saturated iron conditions. Preliminary studies indicate that HFE mRNA expression increases untill cells reach confluence then the level decreases after confluence. HFE mRNA is up-regulated when holo-transferrin was added to the basolateral compartment during 48h before RNA extraction, however the up-regulation is observed only till cell confluence. In conclusion, the iron status seems to play a role in HFE expression before differentiation of intestinal cells that may be involved in the iron sensing to regulate iron absorption.

P0449. The interrelationship between type of the copper nutrition and activity of the genes supporting copper balance in newborn rats during development

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The effect of copper (Cu) nutrition on genes expression controlled the copper turnover during mammal development has been studying. The methods of Northern- and Western-blot hybridization, immunoelectrophoresis, different enzymatic assays and atomic absorption spectrometry were used. A group of newborn rats were fed with baby formula (Cu contains as an inorganic salt) during 8 days from the 1st day after birth (experimental group). The Cu and ceruloplasmin (Cp) levels in their serum were increased 3 times and the liver Cu concentration was decreased 2 times when compared with rats which were fed by rat Dams (Cu packed into milk Cp, control group). The liver Cp-mRNA content was increased and Wilson ATPase gene expression was appeared in liver of the experimental group. The effect of baby formula was distinctly appeared in 48 hours independent of starting fed of newborn rats (from first or eighth day of life). The brain Cu concentration was not changed, but both Cp and Cu levels in cerebrospinal fluid of experimental rats were increased 7 times. Cu was progressively accumulated during first 12 days of life and it was found in nuclei and lysosomes, but its level was already decreased to 5th day of experiment. The Menkes and Wilson ATPases as well as Cp genes expression were mainly expressed in hypophysis and choriodic plexus but not in cerebral cortex and cerebellum of 5-days old rats of both groups.
The roles of milk Cp and Cp gene expression in mammary glad in newborn is discussed.

P0450. Genomic characterisation of the human prion protein (PrP) gene locus

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Prion protein (PrP) is intimately linked with a class of neurodegenerative diseases known as transmissible spongiform encephalopathies, which in humans includes Creutzfeldt-Jakob disease (CJD), Gerstman-Sträussler-Scheinker disease (GSS), Kuru and the recently recognised form of variant CJD (vCJD). Employing bioinformatics and direct molecular analysis we demonstrate that the human PrP gene (PRNP) locus, which is situated at chromosome position 20p12-ter, consists of a functional domain of approximately 55 kb containing three genes; PRNP, DOPPEL or PRND located 20 kb 3' of PRNP and a novel gene, designated HSM8 that maps 3 kb 3' to PRND and which is transcribed to generate at least three alternatively spliced mRNAs. All three genes of this locus show a similar two-exon structure with the protein coding region restricted to the larger exon 2, but low sequence homology implying that although they may be evolutionarily related they are functionally distinct. Analysis of both adult and foetal human tissues confirmed the ubiquitous but variable expression profile of PRNP with highest levels observed in the CNS and testis. Contrastingly, although PRND shows a wide tissue expression pattern in foetal tissues, it is exclusively expressed in adult testis whereas all three HSM8 isoforms were only detected in adult testis implying that PRND is developmentally regulated. An investigation of the regulatory mechanisms underlying this complex gene expression pattern from the PRNP locus should provide insight into the function of these genes and their involvement in prion protein diseases.

P0451. Evolution of the Homologues to the PKD1 Gene

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PKD1 is the first gene responsible for the condition of autosomal dominant polycystic kidney disease (ADPKD) and its gene product is called polycystin 1. There are several homologous genes (HG) to PKD1 which are located proximally to the master gene on the same chromosome. The 3' regions of the HG contain the previously described chromosome 16-specific low-copy number repeat element,

mapped to chromosome 16 p13, p12, and q22. They share a high degree of homology (95% - 97%) to PKD1 over a large genomic segment. We recently showed that these genes are pseudogenes, duplicated in the course of molecular evolution.
It is known that homologues to PKD1 are not present in mouse and lower species. We therefore sought to perform searches for PKD1 homologous sequences in primates in order to reveal at which stage of evolution HG appeared. For this purpose we performed FISH analysis on chromosome spreads from human, chimp, gorilla and orang-utan using BACs containing human PKD1 homologues genes. The expected pattern of signals was obtained by human and gorilla whereas chimp and orang-utan gave additional signals on different orthologs of the human chromosome 7. The orang-utan lacks in addition the signal on the long arm of human chromosome 16 ortholog.
Sequencing analysis of chimp-PKD1 gene revealed more than 99% identity between the master gene and its homologue(s) in the analysed regions. The implication of these results on our knowledge about the evolution of the PKD1 homologues and their possible function in primates will be discussed.

P0452. Simultaneous expression of two murine clustered ADAM family genes, Testase 2a and Testase 2b, during testicular germ cell development .

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The ADAM (A Disintegrin and A Metaloprotease domain) family presents the best characterized candidates for mediating gamete interaction and membrane fusion in mammals. In this study we describe the expression patterns of two clustered ADAM family genes for testase 2a and testase 2b. Previous studies showed that murine testase 2 (ADAMA 25) gene is expressed specifically in testis. However, we found two different restriction patterns of subcloned fragment of the gene, suggesting presence of two novel testase 2 transcripts. Further experiments and Celera database search revealed that these two transcripts are the products of two separate clustered genes, which show high similarity to the published testase 2 (87.8% and 95.4%, respectively) and in 87,4% to each other. Both genes are located on chromosome 8 (as well as ADAM 3, ADAM 5, ADAM 9) in close distance of 24 kb. Genomic structure of testase 2a and b is different from other ADAM family members, like cyritestin or fertilin which possess around 20 short exons. They are composed of only two exons. The first exon is about 85 bp while the second is over 2.5 kb long. Both genes demonstrate the same temporal and spatial expression pattern during testicular germ cell development with onset of expression in haploid stages. Further functional analysis of these molecules will contribute not only to a better understanding of the molecular mechanisms underlying mammalian sperm-egg fusion but also to the development of new methods for both, fertility regulation and diagnosis and treatment of human infertility.

P0453. PKD1 Unusual DNA Conformations are Recognized by Nucleotide Excision Repair.

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The genetic defects in autosomal dominant polycystic kidney disease (ADPKD) cases are mutations in PKD1, a gene that encodes a transcript of 14 kilobases from 46 exons spanning 50 kbp on chromosome 16p13.3. The 2.5kbp poly(purine-pyrimidine)(R-Y) tract in intron 21 of the PKD1 gene may contribute to its high mutation frequency. The rate of somatic mutations must be high given the frequent occurrence of ADPKD and the very large number of cysts observed, suggesting the existence of a local hot spot for mutagenesis. The poly(R-Y) tract is one of the ten longest sequences of this kind; it is 66% G-C-rich with 95% C+T in the sense strand and is partly repeated in introns 1 and 22. The tract contains 23 mirror repeat sequences, which would be expected to adopt three-stranded DNA structures with stems of at least 10 bp. In addition, 163 direct repeat sequences were identified, which may adopt slipped, mispaired conformations. These DNA conformations were recognized as lesions and were cleaved by the NER system. Current work focuses on evaluating the frequency and types of mutations induced by the R-Y tract.

P0454. Cloning of a novel gene on 3q25-26 with homology to nonsense mediated mRNA decay (NMD) proteins via a RA-induction gene trap approach.

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For better understanding retinoic acid (RA)/retinoid (RX) induced teratogenesis we set up an RA-induced gene trap approach using mouse ES clones (Forrester et al. 1996). One of these trapped clones showed repressed RA-induced LacZ reporter gene expression. By 5´ RACE-PCR a fusion transcript was identified which after cloning revealed 1081bp upstream sequences of the integration site. A 1.754 kb cDNA was established from clones identified by screening mouse cDNA libraries. This cDNA was used to isolate a human cDNA by screening a fetal brain Marathon cDNA library. Sequence homology between both cDNAs with an ORF of 1515 nucleotides is 90%. The putative protein contains 503 aas which are 94% identical between both species. This protein shows high homology to NMD3 proteins from yeast species and to proteins conserved in drosophila, caenorhabditis, arabidopsis and leishmania. We isolated a mouse P1 clone and a human BAC clone and established genomic structure of the gene and characterized 5´ and 3´ sequences. The human approx. 33 kb gene contains 15 exons and 14 introns. Northern analysis revealed a 2.5 kb transcript in mouse embryos from day 8-15 pc in heart and craniofacial tissue of newborns as well as in heart and brain of adult mice. Northern analysis of human RNA revealed 2 transcripts of 2.7kb and 3.0kb in heart, liver, pancreas, skeletal muscle and kidney and weaker transcripts in placenta, brain and lung. The novel and potentially RA-repressed gene was FISH mapped to chromosome 3q25q26. (O.B. is a recipient of a DAAD fellowship.)

P0455. A T-box containing transcription factor on human chromosome 15q14 identified through a retinoic acid-induction gene trap approach.

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Prenatal exposure to retinoic acid (RA) and retinoids (RX) is related with specific congenital anomalies. The molecular mechanism for this teratogenesis and the genes involved are not well known. To identify such RA/RX downstream genes we set up an RA-induced gene trap approach based on mouse ES clones (Forrester et al. 1996). From a sample of trapped ES clones with RA-induced reporter-gene repression 5´-RACE-PCR identified a fusion transcript of 1.135 kb in clone EScD1. Screening of cDNA libraries and in silico analysis revealed an approx. 9 kb mouse cDNA. This gene represents a transcription factor containing a T-box and a bHLH Zip-domain. Northern analysis revealed tissue specific expression of this single locus gene with transcripts of varying size. A prominent 9.5 kb transcript is present in spleen, kidney, stomach, lung, brain, testis, ovary, placenta, heart and skeletal muscle. Weaker and smaller transcripts of 7.2 kb were observed in placenta and testis and of approx. 4 kb in testis indicating alternative splicing. A genomic P1 clone isolated contains the complete cDNA. Screening of human cDNA libraries and in silico analysis identified the corresponding human gene with sequence homology >82% to mouse cDNA. BACs were isolated for establishing genomic structure and mapping. The gene contains 24 exons, the same domains as the mouse gene and maps to 15q14, according to FISH. The gene was found to be triplicated in a young proband with mental as well as growth retardation and malformations due to partial trisomy 15q14-q15.

P0456. Characterization of the promoter and new isoforms of CACNA1A gene

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The CACNA1A gene, coding for the α1A-voltage-dependent calcium channel subunit type P/Q, is responsible for Episodic Ataxia type 2 (EA2), Familial Hemiplegic Migraine (FHM) and Spinocerebellar

Ataxia type 6 (SCA6).
Several mutations causing these diseases have been described, and they account for aminoacid substitutions, protein truncations and small polyglutamine expansions. In addition, families segregating the disease with the CACNA1A, but not showing any mutations in the coding region, have also been found. For this reason we started the characterization of the regulating regions of this gene as possible sites for mutations.
Through bioinformatic analysis of sequences upstream the first coding exon of the gene, two candidate regions have been identified. Primer extension experiments have shown a band of about 300 bp suggesting a transcription starting site at 250-300 bp upstream to exon 1. The characterization of this region is ongoing.
The CACNA1A gene shows a considerable complexity of ribotypes. Different isoforms may be associated with different channel activity, as demonstrated in the rabbit. The characterization of different splice variants and of splice enhancer sites will increase the chances to detect non coding mutations in FHM or EA2 patients.
Comparison of different cDNA clones selected with CACNA1A specific probes from an adult cerebellar and a total fetal brain cDNA libraries, revealed the presence of new isoforms. Characterization of isoforms at the 5' end has been performed.
This work was supported by Telethon grant E847.

P0457. Detection Of Rps4x Gene Expression Using Rt-pcr

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Ribozomal protein S4 (RPS4X) gene is located on chromosome X and escapes from X inactivation. Thus, insufficient expression of RPS4X may play a role in development of Turner syndrome, the complex human phenotype associated with monosomy X. In this study we have investigated RPS4X gene expression levels in peripheral blood of 21 patients with 46,XX karyotype and Turner phenotype or its predominant feature primary amenorrhea by RT-PCR, while two 45,X individuals were studied as control subjects to be able to compare the expression levels as we expect lower levels of RPS4X gene expression in 45,X patients due to a single X chromosome.We found different expression levels in 7 of our 21 patients, 6 of which were diagnosed to have primary amenorrhea while 1 had a Turner phenotype, yet observed an increased level of expression in one of the 45,X control patients as well. Based on our results, we debate whether haploinsufficieny of RPS4X is the cause of Turner syndrome or not, and plan to perform sequence analysis to investigate any possible sequence defects which could be responsible for the change in the level of expression in RPS4X gene in our cases. Our results were discussed comparatively with the previously reported data.

P0458. Disruption of KCC2 reveals an essential role of K-Cl-cotransport in synaptic inhibition.

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Synaptic inhibition is crucial for the control and modulation of neuronal activity. Disturbing the interplay between excitation and inhibition causes various neurological disorders. GABA and glycine are the main inhibitory neurotransmitters of the adult central nervous system. Synaptic inhibition by GABAA and glycine receptors, which are ligand-gated Cl- channels, depends on the intracellular chloride concentration ([Cl-]i). High [Cl-]i can lead to excitatory GABA responses that are deemed to be important during development. Several potassium-chloride cotransporters can lower [Cl-]i, including the neuronal isoform KCC2, which was substantiated by antisense experiments in vitro. Analysis of the expression pattern of KCC2 during murine embryonic and postnatal development by in situ hybridization and Western blot analysis, shows that KCC2 parallels neuronal differentiation and precedes the functional GABA switch. Neonate KCC2 knockout (Kcc2-/-) mice die due to severe motor deficits including loss of respiration. Sciatic nerve recordings reveal abnormal spontaneous electrical activity indicating a spastic disorder. Spinal cord responses to peripheral electrical stimuli are altered in Kcc2-/-mice as observed in the mouse mutant spastic. In wild-type animals, immunofluorescence and electron microscopy demonstrated KCC2 expression close to inhibitory synapses. Patch-clamp measurements of spinal cord motoneurons demonstrated an excitatory GABA and glycine action in the absence, but not in the presence of KCC2. This shows that the functional GABA/glycine switch in the spinal cord occurs earlier than in the hippocampus. It depends crucially on the expression of KCC2, and is indispensable for the normal function of motor circuits already at birth.

P0459. The putative mechanisms determining ceruloplasmin gene expression in mammals

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The numerous of tissue specific extracellular molecular forms of ceruloplasmin (Cp, multicopper ferroxidase) and its membrane isoforms: glycosylphosphatidylinositol-anchor Cp, receptor Cp and intracellular membrane Cp-like ferroxidase, play a central role in copper transport through body as well as iron metabolism. Perhaps they are the products of the single Cp gene copy in haploid chromosome number whose mutations lead to various neurodegeneration disorders. In this work the structure analysis of Cp gene promote region (4000 bp upstream transcription start point) was carried out by the original programs for identification and mapping cis-elements potentially taking part in gene regulation. The sequence specific sites for the nuclear receptors of 9-cis-retinoic acid and thyroid hormone and sites for specific protein expression in liver, lung and mammary gland were found and these localization were mapped. The structure and localization of these sites are very similar in rat and human. In vivo experiments shown that estradiol stimulated Cp transcription and translation in the rat liver to 3 folds. The selective interaction of the hormone responsible elements with soluble nuclear proteins isolated from liver and brain newborn and adult rats was demonstrated by gel-shift assays. The similar results were obtained for transcription factors isolated from rat mammary gland during lactation.
The role of Cp gene tissue specific activity in the safe turnover of the copper and iron in mammals is discussed.

P0460. Investigation of chromosomal DNA loop organization within a region of human chromosome 16q22.1

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We have previously generated a 2.8 Mb high-resolution map surrounding the LCAT gene cluster on human chromosome 16q22.1 (Frengen, Rocca-Serra, Shaposhnikov, et al., Genomics 70:273-285, 2000). We suggest that the tight organization of the LCAT gene cluster has biological significance. The domain organization of this chromosome region is currently analyzed by COMET-FISH and by the topoisomerase II-mediated DNA loop excision protocol. COMET-FISH is gel-electrophoresis of DNA from a single nucleus, which is lysed on microscope slide to produce nucleoids. The nucleoids are electrophoresed extending the loops into tails. Subsequent FISH allows mapping of specific DNA sequences/genes from the LCAT region relative to the DNA loops. We are also using a complementary approach, which is based on the ability of cellular topoisomerase II to cleave DNA in the presence of several anticancer agents. The cleavage sites are associated with regions of DNA attachment to the nuclear skeleton, thus indicating DNA loop anchorage sites. Analysis of the topoisomerase II cleavage sites within the region therefore permits mapping of the domain organization. Human cells have been exposed to topoisomerase II inhibitor, the DNA from the cells was cleaved with restriction enzymes, separated by pulsed field gel electrophoresis, blotted, and hybridized with probes from our map. This dual approach allows exploration of the topological organization of the region of human chromosome 16q22.1 and permits correlation of the structural architecture to the functional organization of the DNA.

P0461. A transgenic mouse model overexpressing the human prune gene in epidermis.

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Transgenic mice have proven to be a powerful system to study normal and pathological functions of genes. We have previously reported the isolation and characterization of the human PRUNE gene. Furthermore we have found by "in vitro" experiments that overexpression of prune increases proliferation in NIH3T3 cells. The human PRUNE gene is located in the 1q21.3 chromosomal region, in the epidermis cluster locus where two skin disorders have been mapped: (AD) atopic dermatitis and psoriasis. Prune is expressed in the adult human skin and, in particular, in the basal and granular layers. For these reasons we have cloned the human PRUNE cDNA under the control of a peculiar loricrin promoter, which drive the expression of the transgene both in the undifferentiated and differentiated layers of the skin. Four founders lines have been obtained and morphological studies are in progress by Immunohistochemistry (IHC) analysis with anti-prune Ab, keratinocytes markers (K14, K1, K6) and proliferative markers (example Ki67). BrdU "in vivo" uptake analysis and apoptosis TUNEL assays will be performed to define if prune may interfere with apoptosis. Furthermore previous unpublished results have shown a defect in the skin of SCID mice by the use of retrovirus technology. A dominant negative mutation of prune protein (PRUNE-D) affecting its PDE activity, results in a specific alteration of the cell cycle proliferation and differentiation of the SCID hair follicles. For this reason the mutated prune gene has been cloned under the same promoter and used for the generation of a dominant negative transgenic mouse.

P 8. Genetic Counselling and Genetic Education

P0462. Medical Genetics WebLab

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WebLab is a problem-based on-line course in Medical Genetics, including original simulations of tests and relevant internet links. The course contains >100 problems, and tools to solve them. This is an abridged description. The course is free.
There are four chapters. In 'Cytogenetics' the student finds banded metaphases and FISH results from constitutional, acquired and prenatal cases. A photo-viewer allows to 'cut and paste' the chromosomes in a word document, making a karyotype. Clinical databases put the karyotype in context. The Human Genome is searched for clones suitable for making FISH probes for the regions of interest.
In 'Pedigrees', risk calculation is introduced through a series of pedigrees, including linked markers. A web page calculates risks using Bayes theorem.
The 'DNA Lab' includes a 'virtual freezer' with DNA sequence from families with mutations on the CFTR or FMRX genes. The sequences are not immediately readable but require the use of an original 'virtual electrophoresis lab' where four tests can be carried out on the samples in the freezer; heteroduplex analysis, denaturing gradient gel electrophoresis, sequencing and Southern blotting on double digests (EcoRI and EagI) with probe StB12.3.
Lastly, the 'Protein Lab' contains among other items links to metabolic pathway
charts, 3D viewers, and protein alignment programs. Access to all these tools is made for the purpose of solving specific clinical problems.

P0463. Psychosocial care of lysosomal disorders in Bulgaria

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Psychosocial problems of genetic disorders in Bulgaria are severe and not good known.They were created on the base of their complexity by different diseases , the bad medical information of the Society and the medical doctors sometimes and the stress of the parents.The aim of our work is to investigate the psychosocial problems in families with children with lysosomal diseases.
With the methods of interview and testing there were investigated 58 families of children with mucopolyssharidosis,Gaucher disease,leuc odystrophies.The interview showed that the diagnosis in more cases were late ,the parents worried the delayed physical development of their child,mental retardation ,behavior changes,haemorrhagic diathesis.The parents need multidisciplinary team of specialists for treatment their child.To help the doctors and themself the parents organisated Association for Gaucher disease ,which support the introdussing the Enzyme replacement therapy.The adult patients with Gaucher diseases had not possibility for ERT. The support of bulbar muscle function were the most important problems by children with leucodystrophies
The prenatal diagnosis in these families was very important and support the parents for a new life.The evaluated psychosocial problems of patients with lysosomal diseases will be used for recommendations against Ministry of health and Work and Social care.

P0464. Genetic Counselling for acute infective disease during pregnancy. Experience of a Italian Genetic Centre on 11 years of activity.

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At the Genetic Centre of Trento, from 1990 to half 2001, 1124 Genetic Counselling have been performed about exposure to possible teratogenous factors, 87 cases of which regarding acute infective disease on pregnancy: 46 cases of herpetic infection , 18 of rubella, 11 cases of parotitis, 4 cases of hepatitis, 3 infections of parvovirus, 2 of salmonellosis and 1 case of measles, tuberculosis and borreliosis.
The average age of the woman arrived at the Genetic Centre for acute infective disease was 31 years and 2 months.
Chicken-pox: 41 genetic counselling: 8 virus exposures not followed by disease; 2 induced abortions; 1 spontaneous abortion; 2 new born with congenital malformations; 5 pregnancies still on going; 5 cases lost at follow-up; 20 healthy children.
Rubella virus: 18 genetic counselling; 8 virus exposures not followed by disease; 6 induced abortions; 4 infections not confirmed.
Parotitis virus: 11 genetic counselling; in 2 cases maternal infection has been excluded; 2 women were not pregnant at the time of infection; 5 healthy children ; 2 cases lost at follow-up.
The last 17 pregnancies are resolved in: 16 healthy children and 1 case lost at follow-up.
Conclusions: 1) the most represented average age is quite elevated, since the women were not primipare. Infact, infection disease is mostly transmitted by another child of the couple. 2) rubella virus vaccine reduces the number of epidemic by wild virus, so women not vaccinated have less probabilities to contract disease but more risk in pregnancy.

P0465. The questionnaire to parents of children with the Down syndrome: how to inform the parents and psychological responses to counseling

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This study determined the experience of the 53 sets of parents when they were informed that their child had Down syndrome and how they would have preferred this matter to have been handled.
The survey revealed that the majority of parents would have preferred being told as soon as possible, with both of them present, and that they had suspected something wrong at the birth of the child.
This information prompted us to analyse critically the parental experiences and to formulate a positive approach with sensitive, supportive and progressive counseling.

P0466. Experiences, at the time of diagnosis, of parents who have a child with a bone dysplasia resulting in short stature.

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There has been extensive literature pertaining to the time of diagnosis of a disability and the levels of satisfaction with disclosure of a disability. The information given and the attitudes of the disclosing health professionals during this critical period can have a significant effect on the family. This study explored parents' experience of being told that their child had a bone dysplasia resulting in short stature, and discussed ways of improving this experience for families.
Semi-structured interviews were conducted with 11 families. Families were recruited through the Bone Dysplasia Clinic at the Royal Children's Hospital, Victoria, Australia and via contact with the Short Statured People's Association of Victoria. They were asked about how they were told of their child's diagnosis and what effect that had on them and their families. Parents were asked about how they would have preferred to have been told and what would have made the experience less distressing. Of particular interest to the researchers was the role of information in making the experience less distressing for the families. Transcripts of the interviews were analysed, and major themes were identified relating to the parents' experiences.
We conclude that the manner in which the diagnosis is conveyed to parents plays an important role in their adjustment and acceptance of the diagnosis. The provision of appropriate written information relating to the condition, possible medical complications, positive outlook for their child's future, how to find social services and supports were some of the significant issues for the parents.

P0467. To tell or not to tell: a qualitative study exploring the passing on of genetic knowledge to family members.

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Background: Anecdotal evidence from genetic counsellors suggests that some people tell their relatives genetic risk information, whilst others do not. Similarly people tell some of their relatives, but not others. This issue is important because individuals might be disadvantaged emotionally, socially, financially or medically by having this information withheld or disclosed and conflicts may arise within families if some cannot accept a parent's or sibling's right to privacy.
Methods: In-depth interviews were undertaken with people who have received genetic counselling for risk of Huntington's disease and hereditary breast/ovarian cancer, and their partners. The interviews explored whether relatives had or had not been told their risk by participants; the factors which influenced telling or not telling; who should tell; and views of genetic counselling.
Results: In total, 36 consultands and 19 partners were interviewed. The analysis confirms clinicians observations that people tell some relatives but not others. Data related to telling or not telling different relatives will be presented. Respondents' views about whose responsibility it is to pass on this type of information will be particularly explored.
Conclusions: The level of disclosure to relatives can at times be limited but also depends on the psychosocial, familial and disease context. Disclosure, moreover, should be viewed as a process of telling as opposed to a dichotomy of 'telling' versus 'not telling'. Ultimately, we hope this study will contribute towards a wider understanding into the dynamics within families after someone in that family attends for genetic counselling.

P0468. A study of information needs of primary care physicians (GPs) for management of a rare genetic disorder: Osteogenesis Imperfecta (OI). - Preliminary data

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Does lack of knowledge form an important barrier to primary care management of a genetic disorder, and if so, how can we overcoming it? These questions were addressed using qualitative and quantitative methods.
Parents of all children with OI in Greater London were contacted and their consent requested. From a total of 59, consent was gained from 37 (63%). Details of over 1000 clinical encounters (including telephone) were extracted from hospital and GP notes for a 5-year period. Mean GP contacts were 3.8/yr, compared with 3.2/yr at the specialist hospital clinic. Many (31%) GP contacts were related to the condition, including a wide range of non-fracture problems.
In semi-structured interviews, 65 % of GPs felt that lack of knowledge was a problem in managing these patients. 78% believed that they changed their practice, mainly by referring patients more often or attempting to learn more about the condition. A variety of sources of knowledge were used, the most common being clinic letters, textbooks and the WWW.
When asked to identify features of information resources that made them useful, aspects related to credibility (perception that information is true) were not seen as more important than accessibility or clinical significance. Credibility was assessed by most GPs using authority (from a trusted source) rather than transparency (use of references etc).
These results will be used in a follow up study to construct and validate clinical genetics knowledge resources.

P0469. FOXL2-Mutations in Blepharophimosis-Ptosis-Epicanthus Inversus Syndrome (BPES) - Challenges for genetic counselling of sporadic female patients

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Mutations in the forkhead transcription factor gene (FOXL2) were recently reported to cause blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) types I and II. Evidence was provided that type I BPES (eyelid abnormalities and female infertility) is caused by mutations resulting in a truncated FOXL2 protein. In contrast, extension of the FOXL2 protein is found in type II BPES, in which fertility is generally normal. This genotype/phenotype correlation provides challenges for genetic counselling, as molecular testing may be predictive of female fertility.
We report a 32-year-old female patient with sporadic BPES and a history of menstrual irregularities and periods of secondary amenorrhoea. Mutation analysis revealed a protein extending mutation (c959-960insG) in the FOXL2 gene with a modified sequence for the last 57 amino acids, suggesting a type II BPES despite the menstrual irregularities. The clinical presentation of our patient and of three female patients with type II BPES described by De Baere et al. (Hum Mol Genet, 10, 1591-600; 2001) indicate phenotypic overlap between type I and type II BPES. These observations question clear-cut prediction of female fertility based on molecular results, particularly in sporadic female patients with irregular menstruation and in young women referred for evaluation of facial dysmorphism.
As a consequence, FOXL2 mutation testing in female patients of child-bearing age with BPES should be handled with caution and a two-step genetic counselling approach including a first pre-test information session is proposed.

P0470. Is Genetic Counselling Unbiased? A 36-Nation Survey

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Nondirectiveness has long been the ethically preferred approach in reproductive genetic counseling. Patients are expected to make their own decisions, using unbiased information. To ascertain the attitudes of genetics professionals worldwide toward directiveness in counseling after prenatal diagnosis, we used anonymous mail questionnaire surveys with case vignettes in 12 languages, distributed by colleagues in 36 nations with ten or more practicing medical geneticists (n=4629). Questionnaires presented 21 conditions identified after prenatal diagnosis and asked how the respondent would counsel. 2906 geneticists (63%), including 1084 in US (70%) and 499 US nongeneticist physicians (59%) responded. Except in North America, the UK, and Australia, many geneticists reported that they would be both directive and pessimistic for many conditions, especially in Eastern Europe and Asia. In the UK, 10% would counsel in favor of abortion for Trisomy 21; in Northern Europe, 34%; in Southern Europe, 47%; in Eastern Europe, 63%. For cystic fibrosis, percents were 10%, 33%, 43%, and 63% respectively, and for Huntington disease 10%, 21%, 34%, and 48%. Most regarded educational success of a session as more important than empathy or support; many thought their goal was to prevent the birth of children with genetic conditions, by prenatal diagnosis and selective abortion if necessary. Although internationally recognized ethical standards

support counseling that is as unbiased as possible, it appears that many geneticists would use other approaches. There is a need for global discussion of optimum counseling approaches.

P0471. Evaluating Internet Information on Down Syndrome: Descriptive criteria are not enough

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Because of the huge number of websites, it is difficult to find relevant information on the internet which supports coping with genetic conditions. There are also concerns about the quality of the medical information available in the internet. Therefore, many authors published criteria mainly descriptive ones for rating medical websites. But many problems persist:
Data can change quickly; there is no way to search the internet entirely; it is not clear who should set criteria and control websites; indirect markers of quality like the numbers of links to a site or the number of visitors a day are easy to manipulate. Moreover, it is unknown if such criteria reflect the quality of medical information.
After having conducted a search for “Down syndrome” with two search engines and in two languages (Altavista and Yahoo in German and in English), we investigated 324 hits using a catalogue of medical, psychosocial and formal criteria drawn from pediatric textbooks and litterature.
Formal criteria were more often met than medical or psychosocial criteria. Neither the medical content nor the psychosocial content of websites correlated with the formal content. Beyond that, there were no important correlation between the search engine ranking and the catalogue ranking. However, authorship, language and search engine influenced the information score.
Descriptive parameters are useful but cannot replace an analysis by an expert since they don´t correlate automatically with a website´s medical content. Combining formal description with expert review is necessary.

P0472. The theoretical and normative foundations of genetic counselling - completing a jigsaw puzzle without a picture.

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The speciality of genetic counselling is paradigmatic in many aspects. As a fairly recent member on the medical frontier its practitioners are pioneers of an evolving ethos.
Upon examination of the theoretical foundations of genetic counselling there appears to be an absence of a formal ethical framework. This is further highlighted by the lack of presence of a formal implementation of the four main bioethical principles that are commonly perceived to govern good clinical practice. The normative foundations of genetic counselling rely heavily on the formality that lies within the code of ethics by which a genetic counsellor practises. There is a commendable objective for the counsellor to be adequately trained in order to deliver the best service they can, however, it is not sufficient to have good intentions.
This paper will explore the implications and importance of having a formal ethical framework and whether it can be universally attainable. It will question if regulating the ethics of conduct is a sufficient activity or whether the professionals (genetic counsellors and geneticists) have a parallel duty to define boundaries (and thereby dispelling possible ambiguities and inconsistencies in clinical practice), and debate the desirability (and feasibility) for these boundaries to be independent of the laws of society and public opinion.

P0473. Community genetics in Saguenay-Lac-St-Jean(Quebec,Canada)

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Historical and demographic phenomena explain that certain hereditary diseases are specific to the Saguenay-Lac-St-Jean region (Quebec, Canada) while others, although not peculiar to a region, are proportionally more prevalent there than elsewhere. Myotonic dystrophy and familial hypercholesterolemia figure among the most frequent dominant diseases; cytochrome oxidase deficiency, spastic ataxia of Charlevoix-Saguenay, type 1 tyrosinemia, sensorimotor neuropathy with agenesis of the corpus callosum as well as cystic fibrosis are the recessive diseases which have been observed.
With a view to better understand this problematic and seek solutions to it, parents, researchers and health professionals have regrouped, twenty years ago, to create the Corporation for Research and Action on Hereditary Diseases (CORAMH). This organization’s mission is to promote awareness, information and prevention of these health problems in the population.
To achieve this goal, one of CORAMH’s activity encloses the dissemination of a genetics information program, which consists in presentations on basic notions related to heredity and monogenic diseases present in the region. Every year, through the program, CORAMH provides information to over 2500 youngsters in academic environments. Since the beginning of the program in 1983, almost 30 000 people have received information via the latter.
CORAMH is also the community partner of an important research program called ECOGENE-21: From DNA to the community. In this regard, the corporation plays an essential role in the transfer of knowledge between the universe of scientists and that of the community.

P0474. Genetic Counselling: A case report. A dilemma between Science and Ethics

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Male with 46, XX chromosome constitution is a rare disorder, occurring only 1 in 20.000 newborn males. Affected individuals have a male phenotype, small testes and small phallus. This disorder resembles Klinefelter Syndrome. Approximately 80% of phenotipycally XX males, one of the X chromosomes carries the SRY gene.
The authors present a case of a prenatal diagnosis, which cytogenetic result was 46, XX. The amniocentese was performed at 14 weeks of gestation with the indication of advanced maternal age. The diagnosis was made with the routine GTG banding.
A morphological ultrassonography made at 21 weeks of gestation showed a fetus with a visible phallus and testis, with normal development. Maternal contamination was excluded.
After birth, the confirmation of the cytogenetic diagnosis was done in cord blood, and with FISH studies using the SRY probe- the SRY gene was localized at the Xp chromosome.
How and when should the information be given to the parents? The authors present their perspective in the social and ethics issues and genetic counselling in this particularly case.

P0475. The study of the prevalence of different types of disorders with different proportions of pathogenetic role of genetic factors in families with severe reproductive disorders

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The aim of this pilot study is the ascertainment of different types of genetic load in 307 families with 2482 members of partners with severe reproductive disorders. The three generation genealogy including only first degree relatives did not find any disorders with Mendelian or multifactorial inheritance or solid tumors in 51/307 families (16.6%). In 142/307 families (46.3%) only one disorder was found. In 114/307 families (37.1%) two or three disorders were revealed.
Monogenic diseases were found in 56/2482 (2.26%) of family members with most frequent prevalence of cystic fibrosis and carriers of CFTR mutations (10/2482), (5/2482), different thromboembolic diseases (17/2482). Myotonic dystrophy, polycystic kidney disease, hearing deffects, M. Scheuermann and idiopathic thrombocytopenic purpura were found in 4-7/2482. Multifactorial disorders were revealed in 94/2482 with rather equal proportion of congenital anomalies (48/2482) an other diseases (46/2482). Congenital anomalies of cardiovascular and uropoetic system were

disclosed in 11 - 16/2482 respectively and congenital hip luxation in 10/2482. In multifactorial diseases prevailed immunity disorders (90/2482), cardiovascular diseases (76/2482) and type 2 diabetes mellitus (58/2482). In different types of tumors (89/2482) prevailed tumors of gastrointestinal system (23/2482), breast carcinoma with gynaecologic tumors (31/2482) and tumors of urogenital system (10/2482). The hematopoetic malignancies represented only 7/89 patients with malignancies.
Further study of different types of genetic loads might contribute to the elucidation of their family impact on reproductive disorders and vice versa to the improved genetic counselling and genetic care for families with these problems.
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P0476. Play The Odds : Don't Bet Everything On The Apparent Syndrome

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We have studied two cases referred for multiple congenital anomalies associated with profound deafness. Case 1 : the child was born in 1990 and presented a ventricular septal defect, camptodactyly of the toes, right embryotoxon, bifid uvula with submucous cleft palate and facial dysmorphism. At one year, she presented an acute polyarthritis affecting both large and small joints. At the age of 7 years, she was evaluated in the genetics department. Karyotyping with probe D22S75 showed a 22q11 deletion and the sensorineural deafness, diagnosed at the age of 9 months, was thought to be related to this 22q11 deletion. Case 2 : the patient was born in august 2001, 7th child of first cousins parents. The child was diagnosed with Down syndrome (47,XY,+21) with atrial septal defect. Additional tests were performed for known familial history of vesico-renal reflux (VRR), myopia and deafness. The child was shown to have also VRR, optic nerve hypoplasia and deafness. Despite the context of syndromic associated anomalies, we tested the GJB2 gene (CX26) to evaluate the origin of the profound deafness. Both cases were found to be homozygote for the 35delG mutation.

P0477. The meaning of 'prevention' in the realm of human genetics

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Within the specialities of medicine human genetics is one of the youngest. The introduction of its concepts and its molecular methods has an influence on medical thinking and the use of basic medical concepts. Genetic research is highly interdisciplinary and collaborative which makes necessary the communication between geneticists, various academic disciplines and the public. The informational content of technical terms, which have a (nearly) clear meaning in the realm of medicine in general, is liable to be garbled when transferred to and uncritically used within human genetics. One of the technical terms we focussed on is 'prevention', which e.g. as primary prevention means keeping a disease from occurring at all by removing risk factors but in human genetics especially in prenatal diagnosis is mainly understood as prevention of the ill.
Fourteen scenarios were developed from genetic counselling before conception to abortion of a child with trisomy 21. Three groups of students of medicine differing in their level of clinical and theoretical experience (1st, 3rd and 6th years of medical education) were interviewed using these scenarios in a structured questionnaire. Confounders, concepts of disease and assessment of human genetics in the realm of medicine were recorded.
About 50% of the study group made a distinction between prevention of a disease and 'prevention' of a (genetically)diseased human being, while on the other hand rating human genetics highly responsible for the prevention of genetically caused diseases. The judgement of the different scenarios by the participants was done without a clear concept of 'prevention'.

P0478. Understanding of risk by patients and physicians - how to raise the haze of Bayes?

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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.

P 9. Genetic Epidemiology and Population Genetics

P0479. VNTR Cassette Sequence Diversity In The European R408W-1.8 And R408W-2.3 Phenylketonuria Mutation Lineages

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R408W, the predominant European Phenylalanine Hydroxylase (PAH) mutation in Phenylketonuria (PKU), has arisen by recurrent mutation on chromosomes of haplotypes 2.3 and 1.8. The R408W-2.3 and R408W-1.8 mutations exhibit west-east and east-west clinal distributions across Europe respectively. Crucial evidence for the origin of R408W by recurrent mutation was provided by a previous report [Byck et al. Hum. Molec. Genet. 1994; 3: 1675-1677.], which demonstrated that R408W had arisen on a rare VNTR cassette sequence variant of haplotype 1.8. We have investigated VNTR sequence variation across a range of European populations. DNA samples (n=141) from thirteen European regions were obtained (with appropriate ethical approval) in collaboration with the members of the European PAH VNTR cassette sequence variation study group. VNTR alleles were amplified by PCR, gel-purified and cycle-sequenced in forward and reverse orientation. Cassette sequences were identified according to the standard nomenclature. VNTR-3 alleles exhibited a single cassette organisation (a2-b2-c1) common to all chromosomes whether of wild-type or R408W mutant genotype. In contrast, wild-type VNTR-8 alleles had two different cassette structures represented within the study cohort at roughly equal frequency, namely (a1)5-b3-b2-c1 and (a1)5-b5-b2-c1. R408W-1.8 chromosomes exhibited the (a1)5-b5-b2-c1 VNTR structure alone but were associated with different STR alleles compared to wild-type 1.8 chromosomes. These data confirm the earlier suggestion that recurrent mutation gave rise to R408W mutations on different haplotype backgrounds in Europe and raise interesting questions regarding the relative ages of the R408W-1.8 and -2.3 lineages.

P0480. Haplotype Diversity of the Y-chromosome in Four Mexican Populations

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The non-pseudoautosomal region of the Y-chromosome constitutes a genetic record easily interpretable to obtain valuable anthropological information about the history of worldwide populations. Two bi-allelic loci (YAP and DYS199) and five STRs (DYS19, 389a, 390, 391 and 393) of the non-pseudoautosomal region of the Y-chromosome were analyzed in males from the largest and most widely distributed population in Mexico (Mestizos) and from three Mexican Amerindian tribes: Huichols, Purepechas and Tarahumaras. The allelic distribution of all seven loci was established and it was pairwise compared between populations. For YAP locus, any significant difference ($p > 0.05$) was observed among all four populations. The Amerindian-specific allele DYS199-T was more frequent in Mexican tribes than in Mestizos, establishing the minimum Amerindian component in the Mestizo sample as 18.6%. Tarahumaras were peculiar by its diminished frequency for DYS199-T respecting to Purepechas and Huichols. Mexican Mestizos were different ($p<0.05$) to Huichols, Purepechas and Tarahumaras in five, four and two STRs, respectively. Eighty-eight different haplotypes were observed among the 156 haplotypes obtained. They were grouped in three haplogroups according to the markers YAP (+/-) and DYS199 (C/T): -/C, -/T and +/C. The greater haplotype diversity (D) was observed in Mestizos (98.6 %) and the lower in Huichols (87.17 %). The haplotype variation of the Y-chromosome in Mexican populations was analyzed by AMOVA. The inter-population and intra-population variations were significant ($p<0.0001$) and constituted the 78.5% and 21.5%, respectively. We discuss our findings with previous results about the same populations using autosomal markers (Hum. Biol. 2000, 72: 983-995).

P0481. Human mitochondrial DNA control region sequence variations in Lithuanian population

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The Lithuanians and Latvians are the only two Baltic cultures that survived until today. There are conflicting anthropological findings regarding the process of neolithization in the Baltic region and the formation of the Baltic tribes. However, since neolithic period the native inhabitants of Lithuanian territory have not been replaced by any other ethnic group. Therefore the genetic characterization of the present day Lithuanians may shed more light on the early history of the Balts.
We have analyzed 120 DNA samples from two Lithuanian ethnolinguistic groups (Aukstaiciai and Zemaiciai) using direct sequencing of the first hypervariable segment (HVI) of the control region of the mitochondrial DNA and restriction enzyme digestion for polymorphic site 00073. On the basis of specific substitutions the obtained sequences were classified to mtDNA clusters defined by Richards et al. Sequences of almost all major European clusters (except X) were found in Lithuania. Haplogroup H was the most common mtDNA lineage reaching frequencies from ~40% in Zemaiciai to ~45% in Aukstaiciai. The second most prevalent haplogroup was U, comprising from 25% to 36% of the sequences in Aukstaiciai and Zemaiciai respectively. The frequencies of remaining haplogroups were from 2% to 10%. In general, the mtDNA lineages reflecting more ancient demographic expansion seem to be more frequent, which is compatible with anthropological findings that neolithization in the Baltic region has been largely indigenous process. However, the lineages reflecting the spread of early farmers from the Near East are also present, indicating different processes in the history of Lithuanian population.

P0482. Isonymy, inbreeding and isolation in San Miguel Island (Azores, Portugal): A surname study.

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Small islands constitute isolated populations that offer advantages in disease locus positioning and gene identification. Here we present the population structure of San Miguel island (131 530 inhabitants, 2001 Census), the biggest of nine islands of the Azorean Archipelago (Portugal). Our study was based on surname frequencies that were obtained from the most recent telephone list (2001). We identified 1 315 different surnames in a total of 27 621 subscribers. Eleven places, including the capital (Ponta Delgada) and other rural communities (Achada, Bretanha, Furnas, Ginetes, Maia, Nordeste, Rabo-de-Peixe, Povoação, Salga and Sete Cidades), were chosen according to population size and geographic isolation. Isonymy (I), inbreeding coefficient (Fst), coefficient of kinship between locations (Ri), Fisher's α (α), Karlin-McGregor ν (ν) and Nei's distance were calculated. Salga presents the highest values of isonymy (0.0576) and Fst (0.0144) and the lowest value of α (17.36). Sete Cidades presents the highest value of ν (0.130). Moreover, 51% of Salga's population and 52% of Sete Cidades's population are represented by 6 and 8 surnames, respectively. These results demonstrate the effective isolation and high rates of emigration of these two places, located in opposite edges of the San Miguel island. In contrast, the capital shows the lowest values of isonymy (0.0128), Fst (0.0032) and ν (0.0136) and the highest value of α (78.13). Our analysis suggests a high degree of inbreeding in rural communities of San Miguel island which often constitute a model for genetic mapping studies. (claudia.branco@clix.pt)

P0483. G6PD mutations and UDPGT1 promoter polymorphism among G6PD deficient Kuwaitis

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Glucose-phosphate dehydrogenase (G6PD) deficiency is caused by mutations in the G6PD gene. The clinical manifestation of G6PD deficiency may be influenced by a (TA)n polymorphisn in a promoter of the UDP-glucuronosyltransferase 1 (UDPGT1) gene. The (TA)7 allele is associated with increase in the incidence of neonatal hyperbilirubinaemia in G6PD deficient newborns. To analyze G6PD mutations and UDPGT1 polymorphism in G6PD deficient Kuwaitis, 1080 male blood donors were screened for G6PD activity. G6PD deficiency was identified in 70 (6.5%) individuals. Mutation analysis revealed the Mediterranean (C563T) mutation in 51 (73%), A- (G202A) in 10 (14%), Chatham (G1003A) in 5 (7.1%), Aures (T143C) in 1 (1.4%) of 70 G6PD deficient cases. In three (4.2%) cases mutations remain unknown. Both the Mediterranean and Aures mutations were associated with the C1131T polymorphism while the A- mutation was accompanied by the A376G substitution. UDPGT1 genotyping revealed the (TA)6/(TA)6 genotype in 27 (38.6%), (TA)6/(TA)7 in 31 (44.3%), (TA)7/(TA)7 in 11 (15.7%), and (TA)6/(TA)8 in 1 (1.4%) of 70 G6PD deficient cases. The frequencies of the (TA)6, (TA)7, and (TA)8 alleles among G6PD deficient Kuwaitis were 0.6143, 0.3786 and 0.0071, respectively. The rare (TA)8 allele was observed in a G6PD deficient individual with the A- mutation (both A- and (TA)8 are known to be of African origin).

P0484. Evidence of a founder effect for the tissue-nonspecific alkaline phosphatase (TNSALP) gene E174K mutation in hypophosphatasia patients

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Hypophosphatasia is an inborn error of metabolism characterized by defective bone mineralization caused by a deficiency of liver-, bone- or kidney-type alkaline phosphatase due to mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. The clinical expression of the disease is highly variable, ranging from stillbirth with poor mineralized skeleton to pathologic skeletal fractures which develop in late adulthood only. This clinical heterogeneity is due to the strong allelic heterogeneity in the TNSALP gene. Mutation E174K is the most frequent mutation in Caucasian patients and represents 8% of hypophosphatasia chromosomes. This mutation was found in patients from various geographic origins but was more frequent in the North of Western Europe. We therefore investigated the likelihood of its unique origine or the likelihood of a multiple origin due to recurrence of the mutation on several chromosomes. Three intragenic polymorphisms, S93S, 472+12delG and V505A were genotyped in patients carrying E174K and in normal unrelated individuals. The results show that all the E174K mutations are carried out by a common ancestral haplotype, also found at low frequency in normal and hypophosphatasia chromosomes. We conclude that the E174K mutation is the result of an ancestral mutation that ocurred on a single chromosome in the North of Western Europe and spread

throughout the rest of Europe and into the New World as a result of migrations.

P0485. Population data of Y-chromosomal STRs in the Lithuanian population

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The human Y chromosome is uniparentally inherited and nonrecombining along most of its length. Short tandem repeat (STR) polymorphisms from the male specific part of Y chromosome have been already recognised to be highly valuable in human evolutionary studies and population genetics. Present-day Lithuanians represent the Baltic branch of the populations speaking languages of the Proto-Indo-European descent. Although Lithuanian population was formed under the pressure of various migration forces, its deep roots preserved the genetic composition of the forebears. Therefore, it is reasonable to analyse genetic differences among the Lithuanian ethnolinguistic groups as well as between Lithuanians and other European populations. We present results of the investigation of genetic structure of Lithuanian population by using Y-chromosomal microsatellite markers. We examined the allele and haplotype frequencies of the Y chromosome-specific STR systems (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385) in 57 males from two main Lithuanian ethnolinguistic groups: Aukstaiciai (A) and Zemaiciai (Z). PCR products were detected using capillary electrophoresis on the ABI PRISM 310. In total 44 different haplotypes were identified as a result of combining the 32 alleles of the 7 Y-linked systems containing 9 loci. 35 haplotypes were seen only once, five - twice, four - thrice. The most frequent allele of DYS19 was 15 (52.6%), DYS389-I - 13 (71.9%), DYS389-II - 30 (57.9%), DYS390 - 25 (49.1%), DYS391 - 11 (54.4%), DYS392 - 11 (59.5%), DYS393 - 13 (61.4%), DYS385 - 11/14 (43.9%). Our results were compared with the data of other European populations.

P0486. High resolution analysis of human Y-chromosome diversity in the Western Mediterranean area

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Y chromosome variation was analyzed by surveying 33 biallelic markers in a sample of about 900 males belonging to 23 populations from the Western Mediterranean area. Some populations from the Middle East were also included for comparison. This survey revealed a total of 21 binary haplotypes which were combined with the data from 7 microsatellites to evaluate internal diversities and coalescence ages. The dissection of the YAP+ lineage in several distinct haplotypes showed that only a few derivative haplotypes are present in Europe. One of these haplotypes, defined by the mutation M81, is phylogenetically equivalent to the previously described haplogroup 25.2 (Scozzari et al. Hum Immunol 62:871, 2001). This haplotype is very common in North Western Africa and most likely originated in the Berbers of Morocco where it reaches frequencies of about 70%. The same haplotype is rare in Europe, the only exception being represented by the Spanish from the Pas Valley (43%), thus indicating a strong component of Northern African origin in that region. The haplotype defined by the M26 mutation resulted to be phylogenetically equivalent to the haplotype carrying the 11 repeats allele at the YCAIIb microsatellite. This haplotype is very frequent in Sardinia but is rare or absent in other regions. Overall this study demonstrates that the dissection of Y-chromosome variation into haplogroups/haplotypes with a more restricted geographic distribution can reveal important affinities between populations and provides new clues about their past interactions.

P0487. Multilocus DNA Fingerprinting Analysis as a Tool for Human DNA Divertsity Study

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Over the past years multilocus DNA fingerprinting have been widely applied for the genetic population studies on the different kinds of organisms. We have used this technique with M13 phage DNA as a hypervariable minisatellite probe to investigate 13 human populations from Eastern Europe and Asia. These populations belongs to three language families: Indo-European language family (Slavonic branch: Belarussian), Uralic language family (Finno-Ugric branch: Mari, Mordvinians, Udmurts, Komi), Altai language family (Turkic branch: Bashkirs, Tatars, Chuvashes, Yakuts).
The matrice of all the fragments with the sizes from 10,000-2,000 bp was constructed as a result of the analysis of restriction fragments patterns generated by hybridization. The set of individual patterns was presented as a binary matrice like "Object-Trait".
Level of the population differentiation was estimated according to Lynch (1).
Different statitical analyses (cluster analysis, multiple correspondence analysis, multidimensional scaling) were applied for the treatment of distance matrice obtained from the populations profiles of the fragments frequencies and binary populations matrices as well.
Two territorially separated Belarussian populations produced no regional differnces whereas four separated Bashkir populations appeared to be quite different.
On the whole the correlation between our results and the linguistic affinity data is not absolute.
1. Lynch M. (1990). The Similarity Index and DNA Fingerprinting. Mol. Biol. Evol. 7(5).

P0488. Familial Mediterranean Fever is a frequent disease among Cypriots

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Familial Mediterranean fever (FMF) is an autosomal recessive disease of high prevalence in four ethnic groups, Non-Ashkenazi Jews, Armenians, Arabs and Turks. Typically it presents as acute episodes of periodic fever accompanied by abdominal pain, chest pain, or joint pain. The attack usually lasts from 12 to 72 hours, with arthralgia or arthritis often lasting longer. The most dangerous potential complication is amyloidosis that can lead to ESRF. About 40 mutations have been identified so far, some of them being very frequent. Founder effects have been postulated to be responsible for the high frequency of certain mutations in selected populations. Molecular investigation of the Cypriot population reveals that about 1: 8 is a carrier of one of four mutations, E148Q being the most frequent (1:12). Among 87 MEFV chromosomes analysed, the results are: V726A 27.6%; F479L 21.8%; M694V 20.7%; E148Q 6.9%; M694I 2.3%; R761H 2.3%; Unknown 18.4%. Mutation F479L is rather rare in other populations. Preliminary evidence suggests that this frequent Cypriot mutation is associated with later age of onset of symptoms, the most debilitating of which is strong and frequent abdominal pain, with or without fevers and arthralgias. Despite the high frequuency of E148Q, only 6 of 53 patients carried it, supporting its mild nature. More than have of documented cases were diagnosed during the past year, after announcing the availability of molecular testing and providing evidence that FMF is not rare. Molecular testing is expected to assist further in identifying ambiguous cases, while general newborn screening is under consideration.

P0489. The Incidence of Down Syndrome in Estonia during 1990-2000

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The aim of this study was to investigate the incidence of Down syndrome (DS) in Estonia.
Methods: The data about the children with DS were collected from genetic centers of Estonia, from database of Down Syndrome Supportive Groups, from institutions of disabled children and from the registers of family doctors/pediatricians. The study subjects were 196 DS patients born from 1990 to 2000. Three of them died before

cytogenetic investigation was performed. Therefore 193 children with cytogenetically confirmed DS were included into the study.
Results: Regular trisomy was found in 173 cases (90%), translocation in 14 cases (7%) and mosaicism in 6 cases (3%). In one patient there was regular trisomy 21 and translocation between 13;14 chromosomes. Mosaicism and translocation at the same time (47,XY,t(7;21)(80%)/46,XY(20%)) was found in one child. Thirty percent of the mothers were older than 35 years.
In 1995 screening of chromosome anomalies for advanced maternal age (>35) was started. Therefore we divided the DS children into two groups: born in 1990-1994 and 1995-2000. The first group consist 108 patients (birth-rate: 89015). According to these data the incidence of DS was 1:824. In the second group DS was diagnosed in 85 children (birth-rate: 76767). According to the data of Medical Genetic Center in 32 cases of DS was diagnosed prenatally; in 31 cases the pregnancy was terminated. If those 31 DS children were born the incidence of DS in the second group would be 1:662.
In conclusion we may say that the provisional incidence of DS is 1: 700.

P0490. Y-chromosome haplotype analysis in three Eastern Europe populations - Belorusia, Russia and Ukraine.

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Y-chromosomal microsatellite haplotypes are highly valuable in human evolutionary and human history populations studies. Distribution of Y-chromosome haplotypes for 5 microsatellite loci (DYS393, DYS392, DYS391, DYS390 and DYS19) was investigated in three population- Belorussia, Russia and Ukraine to reconstruct the evolution of paternal lineage in populations with Slavic origin from East Europe.
To determine affinity between Belorussian, Russian, Ukrainian and other populations comparative analysis of allele frequencies was performed and Neighbour-Joining tree based on Nei distances was constructed. So, highly significant differences were observed when comparing the populations from East Europe with populations from West Europe. But although the Y-chromosome microsatellites seem to be very useful in comparing closely related populations, we have not found significant differences between Ukrainian, Russian and Belorussian populations.
For more than 360 Y chromosome assuming a stepwise mutation model haplotypes net was constructed. The three populations tested had significant differences in their haplotypes distributions. The most common haplotype (13/11/11/24/16) was found in 14% of Ukrainian individuals. In population from Russia the most common haplotype (13/11/10/25/16) was observed in 22% individuals. For population from Belorussia both of these haplotypes were most frequent - 13% and 10% respectively. Also it is interested to note that these haplotypes in Western European populations are to be found with very low frequency. On the other hand, the frequencies of these haplotypes in some Central Asian populations reaches extremely high level - up to 50%. . Perhaps, male-lineage diversity in Slavic populations underdone of migration influence from East.

P0491. Epidemiological surveillance of congenital anomalies in north-western Croatia

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BACKGROUND: Birth defects are the most important cause of perinatal mortality in European countries. International standardised networks of registries of patients such as the Eurocat allow data comparison and give the possibility of gaining experience in surveillance and prevention of rare diseases. AIM: To to determine population-based prevalence rates, types and epidemiological characteristics of the major congenital anomalies in northwestern Croatia using standardised Eurocat methodology. RESULTS: During the 1990-1999 period, 1274 children with congenital anomalies per 64.364 births were registered, with the mean prevalence of 19.7/10 000 births. Stability in the overall prevalence of malformations was observed, but there were differences in congenital anomaly rates between different regions ($p<0.01$). Limb anomalies (32.3%) and congenital heart defects (24.2%) represent the largest groups of anomalies detected, followed by the defects of the urogenital system, gastrointestinal system and central nervous system. An unusually high rate of polydactyly was observed in Varazdin (16.9/10000 compared with mean rate of 8.0/10000 for EUROCAT registries). The most frequent congenital heart defects were ventricular septal defect (14.5/ 10 000), atrial septal defect (8.4/10 000) and transposition of the great vessels (2.2/10 000). Down syndrome was the most frequent chromosomal aberration (10.7/ 10.000 births). CONCLUSIONS: Differences in congenital anomaly rates between different regions of Croatia require further investigation in order to determine whether they represent a true difference in the prevalence rates or they are due to small number variation, differences in clinical reporting or characteristics of the population (e.g. size, maternal age).

P0492. Allelic associations of COL1A1 and VDR3 genes with one-year rates of bone mineral mass loss in postmenopausal women.

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Genetic factors play an important role in the pathogenesis of osteoporosis but the genes that determine susceptibility to pathological BMD are still not fully determined.
The alleles rates of COL1A1 and VDR genes in North-West Russian population and in the group of the postmenopausal women were investigated. The control group included 174 women without any clinical or laboratory traits of osteoporosis. The group of patients comprised 119 postmenopausal women (all of them had been in menopause for 4-5 years). Respective of BMD loss speed. two groups of the postmenopausal women were allocated after 12 months survey: women with fast loss of BMD (T-criteria decrease to 10±6,38%) and women with slow loss of BMD (T-criteria decrease during 12 month (2±8,23%). The polymorphisms of COL1A1 and VDR genes were studied by PCR-RFLP method (polymorphism COL1A1/Apa I and VDR3/Taq I).
The differences in allele combinations of VDR and COL1A1 genes between control group and the group of postmenopausal women with osteoporosis were proved. Frequencies of functionally impaired allele of COL1A1 gene (s allele) was 17,9% (control group), 2.4% (patients with slow loss of BMD), 30.8% (patients with fast loss of BMD). Frequencies of functionally impaired allele (t -allele)of VDR gene was 32,7% (control), 13.9% (patients with slow loss of BMD), 46.8% (patients with fast loss of BMD) . Significant association of BMD loss values with functionally inferior alleles for COL1A1(s) and VDR (t) genes has been proved ($p<0,001$).

P0493. PedigreeQuery: a pedigree drawing software.

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Modern human genetics data includes large (hundreds of people) pedigrees coming, in particular, from isolated populations. It is a problem to draw these pedigrees and to analyze the pictures due to their complex structure. Our software PedigreeQuery is based on step-by-step drawing in which a nuclear pedigree is added at each step. The direction of a pedigree extension is indicated by the user. The software helps visualizing the transmission of rare alleles, extracting the fragment of complex pedigree with identical mitochondrial genome or identical Y-chromosome.
The software uses a data file with LINKAGE format and creates PostScript files for printing. PedigreeQuery draws complex pedigrees with multiple loops, inter-generation mating, individuals with multiple mates. There are several additional features for visualization of genetic data: different colors of symbols according to the different phenotypes, crossing symbols, text underneath the person's symbol which includes any desired information (genotype markers, name, age, etc.). Drawing pedigrees is highly configurable and may be specified by nine parameters, i.e., font and symbol's size, space between symbols, and others.
This software can be widely used both in population genetics and genetic epidemiology.

P0494. Genetic polymorphism in Cumanian population determined by analysis of ancient bone samples

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Between 1975 and 1999 thirty-nine tombs were discovered in Csengele (Hungary). The archeologists presumed that the buried persons belonged to the Cumanian ethnic group who, according to the historical data, settled down in that area of the early Hungary during the XII-XIII centuries. The anthropological analyses of the Cumanians suggest that they might be originated from Asia, however their genetic origin has not been known.
DNA was extracted from 11 bones for studying the genetic origin of Cumanians. From the extracted DNA gender determination and mitochondrial D-loop sequence analysis has been carried out.
Using X and Y chromosome specific alphoid satellite markers the gender of the 6 bone samples out of 11 were determined. Our analysis confirmed the anthropological sex identification of four adult individuals as males and was successful in cases of the female juvenile individuals. The remaining five samples did not preserve the investigated genomic DNA markers in detectable form.
The nucleotide sequences of the mitochondrial hypervariable region I were determined. The genetic homogeneity was investigated within the studied Cumanians, and the phylogenetic relationship among the Cumanian samples and modern populations was also established. One part of the investigated Cumanian bone samples carries common maternal lineages with the Asian Mongol and Buryat populations, while the other part shows common mitochondrial haplotypes with some modern European populations. We postulated that the Cumanian population originated from that area (putative homeland) where the modern Buryat and Mongol populations live, at that time when they reached Hungary they were not genetically homogenous.

P0495. Search for Factor V Cambridge and Hong Kong mutations

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The presence of mutations of the factor V gene such as Arg306Thr (factor V Cambridge), Arg306Gly (factor V Hong Kong) and Arg506Gln (factor V Leiden) were tested in 1360 Hungarian thrombotic patients and in 670 controls. We examined 553 hematological patients (e.g. deep venous thrombosis, pulmonary embolism) and 807 patients with different neurovascular diseases (including stroke, ischaemic cerebral attacks, sinus thrombosis, cerebral infarctions). For the FV Cambridge and Hong Kong mutation tests a PCR-based method was developed in our laboratory. After amplification of a 252 bp fragment of the gene, it was digested with MvaI. Both the above mutations abolish the cleavage site for this enzyme. Further differentiation was performed by digestion of the original PCR product by HpaII. For the factor V Leiden mutation we found 111 heterozygotes and 6 homozygotes in the hematological group (allele frequency:11.1%), 83 heterozygotes and 5 homozygotes in the neurovascular cohort (5.76%) and 43 heterozygotes and 3 homozygotes among the controls (3.66%). The allele frequencies of the Leiden mutation showed statistically significant difference in the disease groups versus the controls. In contrast to our expectations neither the factor V Cambridge nor the factor V Hong Kong mutations could be detected in the above mentioned groups. Our findings suggest that the FV Cambridge and Hong Kong mutations are even more rare than previously supposed and cannot be significant contributors to the APC resistance related thrombophilias.

P0496. Microsatellite DNA variability in the dystrophin gene in three Asian populations

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In order to establish the informativeness of polymorphic markers in the dystrophin gene for linkage analysis in our local ethnic groups, we analysed seven multiallelic STR loci in normal X chromosomes from Han Chinese, Malay and Asian Indian populations. The markers examined were the 3‘ flanking DYSI, intragenic STR44, STR45, STR50, STR62, STRHI, and the 5‘ flanking DYSIII microsatellites located at Xp21. Allele frequencies, heterozygosity values, and pair-wise population differentiation were calculated using the GENEPOP (version 3.1d) software. The number of alleles ranged from 4 to 15, with the highest and lowest average heterozygosity values observed at STR44 (87%) and STR62 (34%), respectively. Genetic differentiation was observed between all population pairs at STR44, STR62, and STRHI. The allele frequency distributions at all loci showed varied patterns: DYSI, STR62 and STRHI showed unimodal patterns while DYSIII was bimodal; STR45 showed complex, bimodal, and unimodal patterns while STR44 and STR50 showed complex patterns in all populations. Small to significant differences were seen between the data observed in our populations and those reported for other Asian and Caucasian populations. Genetic heterogeneity found for the microsatellite loci demonstrate that these markers are effective tools for linkage analysis for non-deletion cases of Duchenne and Becker muscular dystrophy families, as well as for anthropological studies.

P0497. Extent of linkage disequilibrium in Sardinian population.

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There is evidence that Linkage disequilibrium (LD), the non random association of alleles at closely linked loci, vary between populations. As the feasibility of linkage disequilibrium mapping studies are critically dependent on the extent of linkage disequilibrium, we examined the patterns of LD for 24 polymorphisms in a sample from Sardinian population. Founder haplotype sets were identified by Simwalk2 program in a sample of 100 families. Microsatellite markers came from three genomic regions - 118 Kb on chromosome 12q14, 10 Mb on chromosome 13q14.2-q21.1 and 128 Kb on chromosome on chromosome 16p12.1-p11.2. For each marker pair, the strength of association was measured by a chi-square test and the standardized multiallelic D’ was calculated. A useful measure is the half-length of LD - the distance at which the average D’ drops below 0.5.
As expected, LD tended to be most significant for nearby markers, but occasionally markers at 6 - 6,5 Mb apart showed $p< 0.05$. At distances up to 200 Kb, all the marker pairs LD but one (95%) appeared significant ($p<0.05$). The negative correlation between D’ and physical distance was striking and significant ($rs = -.625$, $p<0.01$). Average D’ value was 0.51 at distances up to 50 Kb, but it dropped to 0.28 at distances up to 100 Kb. We are at present extending this study, genotyping other markers in the same and another genomic regions. These results, if representative of the whole genome, would suggest the feasibility of whole-genome association studies in Sardinian population.

P0498. Mutation A1555G in the 12S rRNA gene and its epidemiological importance in German, Hungarian and Polish patients

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The A1555G mutation in the 12SrRNA gene has been associated with aminoglycoside induced and nonsyndromic sensorineural hearing impairment. In this study we analyzed Hungarian, Polish and German patients with nonsyndromic severe to profound hearing impairment of unknown origin for this mutation. The frequency of the A1555G mutation in the Hungarian hearing impaired population was below 1.8 %. Three out of 125 Polish patients carrying the A1555G mutation were identified. Among German patients one carrier was found (0.7 %) revealing a homoplastic A1555G mutation, whereas

no mutation was detected in control individuals with normal hearing (frequency < 0.6%). In summary the frequencies of the A1555G mutation are low in the hearing impaired as well as in the normal population in Hungary, Poland and Germany. Since the importance of this mutation and its relationship with aminoglycoside exposure is not well understood yet, patients with nonsyndromic hearing impairment should be routinely screened for this mutation to avoid aminoglycoside induced hearing impairment due to increased sensitivity of maternal relatives.

P0499. NAT polymorphisms in Bulgarian patients with Balkan Endemic Nephropathy (BEN)

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NAT enzymes are involved in metabolic activation and deactivation of environmental carcinogens, such as arylamines. Several allelic variants determine the phenotype of rapid or slow acetylators. Each phenotype has been associated with different risk to certain cancers, related to carcinogenic exposure. Various environmental carcinogens are suspected to be etiological factors for the chronic nephritis and for the frequent development of uroepithelial (bladder, renal pelvis and ureter) tumours in BEN patients. There is a lack of data on NAT allelic frequencies in BEN patients and in the healthy Bulgarian population. 72 Bulgarian BEN patients and 112 healthy Bulgarians as controls were genotyped for NAT1 (C1095A, T1088A, C559T, G560A, T640G) and NAT2 (T341C, C282T). Rapid cycle PCR and melting curve analysis were used for genotyping. To increase the throughput of genotyping probes were designed for temperature multiplexing where possible. The estimated frequency of the predictive NAT2 phenotype for the rapid acetylators was 0.486 in BEN patients group versus 0.464 in the control group and for the slow acetylators 0.514 versus 0.536 respectively. The frequency of the predictive NAT1 phenotype for the homozygous normal acetylators was 0.639 in BEN patients group versus 0.580 in the control group; for the homozygous rapid acetylators 0.042 versus 0.071; for the heterozygous rapid acetylators 0.292 versus 0.339 and for the heterozygous slow acetylators 0.028 versus 0.009. Homozygous slow acetylators were not identified. Allele variants and frequencies did not significantly differ between the two groups and were similar to those reported for the Caucasian population.

P0500. Interest of haplotypic tests to detect the role of a candidate gene in multifactorial diseases

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Background: When studying the potential role of a candidate gene in a multifactorial disease, information may be not available on the functional marker, but only on two neutral markers which are in linkage disequilibrium with the functional one.
Objective: To compare the power of haplotypic tests, taking into account simultaneously the information on two neutral markers, to the power of tests realised individually on each marker.
Methods: A program was written to calculate for patients and controls the frequency of haplotypes and alleles for the two markers, according to different models for the functional polymorphism (allelic frequency, different genotype penetrances) and different values of linkage disequilibrium. Power was then calculated for the haplotypic test and the tests on each marker.
Results: In many situations, the role of the candidate gene is detected by the haplotypic test when it can not be detected by the separated study of each marker. The power increase of the haplotypic test depends on the contrast of the linkage disequilibrium between patients and controls, disequilibrium which may be sometimes of contrary sign in the two samples. This situation was observed in the study of the low density lipoprotein receptor-related protein gene in Alzheimer's disease (Verpillat et al, 2001).
Conclusion: In some situations, the use of simultaneous information on two markers of a candidate gene, using haplotypic tests, can strongly increase the power to detect the role of this gene.

P0501. Insertion-Deletion Polymorphism of the Ace Gene in Populations of North Caucasus and of Middle Asia

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Polymorphic Alu-repeats (polymorphisms consisting of the presence/absence of an Alu element at a particular location) present a very useful markers for human population genetics and evolution studies. A well studied polymorphism in the intron 16 of the angiotensin-converting enzyme (ACE) gene due to insertion (I) or deletion (D) of a 287 bp sequence has been reported to occur producing three genotypes DD, DI and II.
We have investigated the frequency of ACE insertion/deletion (ID) polymorphism in two populations of North Caucasus : Cuban Nogays from republic Karachaevo-Cherkesia (n=102) and Caranogays from republic Dagestan (n=121) and in two populations of the Middle Asia: Kazakhs (n=168) and Uzbeks (n=105). Caucasus and Middle Asia are an interesting regions for studying relative influence of linguistic variability and geographic barriers on the genetic structure of populations.
We used PCR method. PCR product was subjected to polyacrilamid gel electrophoresis. ACE genotypes frequencies for DD, ID and II were determined 19,6%, 58,3%, 22,1% in Kazakhs and 12,4%, 73,3%, 14,3% in Uzbeks and 24,5%, 58,8%, 16,7% in Nogays and 23,9%, 67,8%, 8,3% in Caranogays. The ACE allelic frequency distribution for D and I alleles was 48,8% and 51,2% in Kazakhs, 49% and 51% Uzbeks, 53,9% and 46,1% in Nogays, 57,8% and 42,2% in Caranogays.
Results of our investigations as compare to analoges of literature data.

P0502. The mutation spectrum of CFTR gene in the CF patients from Bashkortostan.

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Cystic fibrosis (CF) is one of the most common autosomal recessive disorders. A mutations CFTR gene causes this disorder. The aim of our study was to identify the most common mutations and create a CF-screening programme that would enable complete CF mutations detection among our patients. We have screened 141 CF chromosomes for 18 previously reported mutations: delF508, 1677delTA CFTRdele2,3(21kb), 394delTT, 1154insTC, R347P, R334W, G542X, G551D, R553X, S549N, 2184insA, 2143delT, S1196X, 3737delA, 3849+10kbT C, W1282X, N1303K. We also used SSCP-analysis for exons 3, 7, 10, 11, 13, 19 and 20, to find other mutations. The results of molecular analysis revealed the following frequency of detected mutations: delF508 (34%), 394delTT (3,5%), CFTRdele2,3(21kb) (1,4%), R334W (1,4%), G542X (0,7%), 2184insA (0,7%), S1196X (0,7%), 3849+10kbT>C (1,4%), W1282X (0,7%), N1303K (1,4%). Six novel mutations are detected I488M (0,7%) Q493E (0,7%) in 10 exon, 1811+12A C (1,4%) - 11 intron, T663S (1,4%) - 13 exon, I1226R (0,7%) -19 exon, 4005+9A>C (0,7%) - 20 intron. And two new polymorphisms was found - 2097A C (A655A) -13 exon (0,7%), 3996G>C (V1288V) - 20 exon (2,1 %). This leading study resulted in the identification of 16 different mutations accounting for almost 50% of CF alleles.

P0503. Mitochondrial DNA diversity in the populations of Middle Asia and Northern Caucasus.

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Hypervariable segment 1(HVS-1) sequences of mitochondrial DNA together with RFLP sites diagnostic analyzed in the populations of Middle Asia - Uzbeks, Kazakhs and population of Northen Caucasus - Nogays in order to determine the haplogroup structure. The

Nogays was found to have a large portion of mtDNA belonging to haplogroups observed in West Eurasian populations. The majority of the mtDNA lineages of Nogays belong to haplogroups H (23,6%) and U (20,2.%). East-Asian-specific lineages of mtDNAs were observed in 38,2% (haplogroups M,C,Z,D,G,A,B,F). On the data of mtDNA genetic variation we demonstrated that the Nogays have slightly more similarities with South-Eastern Bashkirs, which live in the Volga-Ural region of Russia than with other populations from Caucasus. The most maternal lineages of Kazakhs and Uzbeks have the Asian mtDNA haplogroups. However, the relative frequencies of this continental fraction of the mtDNA pool vary considerably over the populations studied. The frequency of Asian haplogroups in Kazakhs reached 62,38%, in Uzbeks - 52,52%. Thus, based on the mtDNA haplogroups composition analysis, the Kazakhs and Uzbeks were found to be the closest population to the East Asian populations.

P0504. Phylogeography of Y chromosome lineages in the Old World

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We have analyzed the distribution of Y chromosome haplogroups (HGs) in the Old World with the special attention to the population of North Eurasia. We have constructed the database containing data on Y chromosome lineage`s frequency in 300 world populations including our experimental data on 30 populations of Siberia and Central Asia and all data published in the literature. We standardized the HGs nomenclature used by different authors to the unified format (Jobling et al. (1998), with modifications). Graphic representation of HGs distribution was made using Surfer software.
The general picture of the Y lineages distribution is consistent with the hypothesis of recent African origin of modern human. Several HGs marks the following migrations of modern human to Europe, Asia, Pacific and colonization of the New World.
As to population of North Eurasia, phylogeographic analysis reveals several components of different age and origin in their modern Y lineages pool, penetrating the territory through boreal and southern migration routs. Initial colonization of this territory, probably, through West, Middle and Central Asia in upper Paleolithic brings to the territory HG2 and 4. A next Paleolithic migrations from the West is traced by HG1. After last glacial maximum, mongoloid populations from the South, descendants of “austratic population” which settled the South-East Asia, penetrates the North Eurasia. The genetic traces of these migrants in male gene pool are, probably, HG10 and 26. Population migrations on the territory of North Eurasia in the Neolithic form the distribution picture of HGs 3, 9, 12 and 16.

P0505. Incidance of Genetic Thrombophilia in Turkish Women with Obstetric Complications-Preliminary findings

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Background: Venous thrombosis is related with some mutations like Factor V Leiden (FVL) and prothrombin 20210A. Obstetrical complications such as preeeclampsia, IUFGR and some others are known to be associated with placental thrombosis. This study investigates the relation between certain gene mutations and obstetrical complications in Turkish women.
Methods: 88 pregnant women with obstetrical complication and 44 control subjects were studied. FVL, prothrombin 20210A and MTHFR C677T mutations were screened using PCR-RFLP method.
Results: Results have yet been obtained for 54 patients and 44 controls. The incidance of FVL heterozygosity was found to be 20.37% among patients, being most common in preeclamptics (7/34) followed by stillbirth (2/9) and IUFGR (2/10) groups. Only 2 stillbirth cases of 54 patients (3.7%) carry both the prothrombin 20210A and FVL mutations. Of 22 patients screened for MTHFR C677T mutation, 12 (55%) were found to be heterozygous carriers whereas the ratio of homozygotes is 9%. (2/22, both preeclamptics). In the control group, FVL mutation was found in 6.82% of the cases although prothrombin 20210A mutation was absent.
Conclusions: The study being still progressing, FVL gene mutation seems to be relatively common in pregnancies with preeclampsia than the uncomplicated ones. Both prothrombin 20210A and MTHFR C677T mutations were detected in certain groups, but the number of subjects is not sufficient for reasonable statistical analysis. However it looks likely that the suggestion coming out of some other studies to screen women with complicated pregnancies for thrombophilia markers can be supported.

P0506. Unusual frequency of the GSTM1 0/0 genotype in a Russian population of Ural region

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Glutathione S-transferase M1 (GSTM1) belongs to the GST gene family. GSTM1 enzyme is responsible for the detoxification of active metabolites many potential carcinogens like trans-stilbene oxide, benzopyrene and other polycyclic aromatic hydrocarbons (PAH). Therefore GSTM1 may be important in modulating susceptibility to cancers. GSTM 1 exert part of glutathione peroxidase activity and have an important function in intracellular binding and transport of a wide variety of endogenous (steroid hormones) and exogenous (drugs) compounds. GSTM1 is polymorphic, and the null alleles result in a lack of corresponding enzyme activities. The presence of the GSTM1 null genotype is responsible for the highest level and significant induction of chromosomal aberrations.
The GSTM1 genotype was analysed from blood spots by means of PCR assay. Mutation in GSTM1 gene was investigated in a random sample of healthy subjects from villages of Ural’s region (around Ekaterinburg-city), Russia
All the results were divided into 2 groups: GSTM1-positive (included GSTM1 1/1 and 1/0 genotypes) and GSTM1-negative (GSTM1 0/0 genotype). Frequency of the GSTM1 0/0 genotype in our study was more than 60%. This unusually high proportion of GSTM1-null genotype among healthy individuals could be attributed to the possible founder effect in villages which have stable populations. Also Ural region is the place where mix Caucasian and Asian populations On the other hand, It might reflect real geographical-dependent differences among Slavic populations.

P0507. Minisatellite DNA diversity in East European populations.

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Most of the populations of Eastern Europe inhabit an area of long time relations between Caucasoid and Mongoloid peoples. This makes it possible to analyse the interaction during ethnogenesis of European peoples. The normal variability of minisatellite loci D1S80 (pMCT118) and 3’ApoB have been analysed in some East European populations. There are both Caucasoid populations (Belorussians, Russians) and admixture populations with different levels of a Mongoloid component (Komis, Mari, Bashkirs). Kalmyk population, belongs to Mongoloid group, was also considered. More than 1200 native individuals from 14 population samples were studied.
The analysis of minisatellite polymorphisms was carried out using the PCR and subsequent electrophoresis followed by silver staining. We detected 27 alleles of the D1S80 locus and 24 alleles of the 3’ApoB locus. Observed allele frequency distributions in Russians, Belorussians, and Mari were appeared to be similar with European populations ones. Minisatellite allele frequency distributions in Kalmyks are very special but more similar with distributions in other Asian peoples.
Genetic diversity analysis based on calculation of Nei’s genetic distances with allocation of populations studied in multidimensional

space. The plots obtained revealed certain differentiation between East European ethnic groups. In spite of the patterns similarity, the total genetic variability of the 3'ApoB locus changes more extremely in comparison to total variability of the D1S80 locus. The genetic diversity of Komis and Bashkirs indicate a number of peculiarities that supposed to reflect an intermediate position of these ethnic groups between Caucasoid and Mongoloid peoples.

P0508. Mitochondrial DNA analysis in Poles and Russians

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Mitochondrial DNA (mtDNA) control region sequences were determined in Poles (n = 436) and Russians (n = 201). Despite the high mtDNA diversity, both populations are characterized by similar pattern of mtDNA haplogroup distribution, which is also typical for many European populations studied. The analysis of mtDNA haplotype distribution has shown that both Slavonic populations share them mainly with Germans and Finns. The following numbers of the rare shared haplotypes and subclusters were found between populations analyzed: 10% between Poles and Germans, 7.4% between Poles and Russians, and 4.5% between Russians and Germans. A novel subcluster U4-310, defined by mutation at nucleotide position 310 in HVS II, was found predominantly in common between Poles and Russians (at frequency of 2%). Given the relatively high frequency and diversity of this marker among Poles and its low frequency in the neighbouring German and Finnish populations, we suggest a central European origin of U4-310, following by subsequent dispersal of this mtDNA subgroup in eastern European populations during the Slavonic migrations in early Middle Ages. This work was supported by the Russian Fund for Basic Research (00-06-80448) and by grant from the Ludwik Rydygier Medical University in Bydgoszcz (BW66/2002).

P0509. Population structure of Adygs, as revealed using DNA data and surname frequencies.

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Adygs people represents indigenous population of Caucasus and belongs to the North Caucasus linguistic family. The ethnos is strongly subdivided into four tribes, each consists of some smaller groups. The most peculiar tribe is Shapsugs, which is divided from other Adygs by main Caucasus mountains chain. Analysis of inter- and intra group genetic diversity has been performed in the hierarhical population system: ethnos - tribes - villages.
Surname frequencies were obtained almost totally for all Adygean local populations (61 villages, near 60000 individuals). Nuclear DNA markers (ApoB and D1S80 minisatellites) and mtDNA markers (HVR1) have been analysed. More than 400 individuals have been studied in total.
We detected more than 16 alleles for each minisatellite loci. The geterozigosyty values are: 0.72 (D1S80) and 0.85 (3'ApoB) in Adygs; in Shapsugs 0.76 and 0.67 accordingly. As for HVR1 mtDNA, in Shapsugs 64 mitotypes and in other Adygs 80 mitotypes have been revealed (sample sizes are 107 and 239).
Fst level of intergroup diversity in whole population system of Adygs as revealed by surname data amount to 0.0209. Different tribes appeared to be quite different by Fst levels: level varies from 0.0025 for Kabardinians to 0.0253 for Shapsugs. Since, strong genetic heterogeneity has been revealed in population system of Adygs. The work was supported by RFBR grants.

P0510. Allelic association of gene markers in the FC epsilon receptor I beta gene and IL-4 gene promoter in Italian atopic children.

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It is known the correlation between the FC epsilon receptor I beta(FCERIB) gene and IgE levels.Indeed,the beta-chain of the high-affinity receptor for IgE is found on mast cells and basophils.Crosslinking of this receptor leads to increased IL-4 production by these cells.Mutations in the FCERIB gene could alter IL-4 production and thus modify IgE levels.IL-4 is essential for B cells switching to IgE antibody production and for maturation of T-helper cells to the Th2 phenotype.A panel of 100 children(58 girls and 42 boys) with atopy was selected from 24 families of Sicily (Southern Italy).A group of 103(50 girls and 53 boys) nonatopic controls included outpatients with no history of allergic diseases was recruited.Atopy was defined by the presence at least of two of following criteria:1)a positive skin prick test;2)elevated specific IgE at least class 2;3)elevated circulating total IgE.In both groups,we have performed the molecular screening of FCERIB gene GLU237Gly and IL-4 promoter C-590T polymorphisms(RFLPs)by allele-specific PCR and PCR-SSCP.Significant difference was observed in the genotype frequency at codon 237 of the FCERIB gene between atopic children and not atopic controls (p=0.044).This RFLP was also associated to elevated levels of IgE,positive prick and rast tests.There was no difference in genotype frequency of IL-4 promoter -590C/T between the two groups.No associations were observed between this RFLP and total IgE and allergic tests.The results of our study suggests that genetic markers of atopy can be identified and could be used in the clinical setting to identify individuals at risk for atopy.

P0511. Does accounting for gene-environment (GxE) interaction increase the power to detect the main effect of a gene in a multifactorial disease ?

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Despite tremendous efforts, few genes involved in the susceptibility for complex disorders have been identified. One explanation is that these disorders are a result of an interaction between genes and environment, and under such conditions, it may be difficult to measure the true genetic effect without accounting for the interaction. Umbach and Weinberg (2000) have proposed an association test which looks at the joint effects of genotype and environment using case-parent trios. In this study, we explore under which conditions accounting for GxE interaction enhances one?s ability to detect the main effect of the gene. Using asymptotic power calculations, we investigate the power to detect the gene effect over varying exposure frequencies and several models of GxE interaction. We show that, for a given sample size, interaction model and allele frequency, the gain in power while accounting for the interaction depends on the exposure frequency : the largest gain are seen for the smallest exposure frequencies, and a loss of power can even be observed when the exposure is frequent and/or the exposure effect is strong. If we consider a gene with a disease allele frequency of 0.2, with no effect in the absence of exposure, an exposure with a 10-fold increase risk and a GxE relative risk of 2, then : when the exposure frequency is 0.1, accounting for GxE interaction increases the power to detect the gene effect in 200 trios by 10%; when the exposure frequency is 0.9 it decreases the power by 15%.

P0512. Mutation spectrum of CAPN3 gene in LGMD2A patients in Croatia

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Background. Our previous results have shown that the most frequent mutation in CANP3 gene in patients from Croatia is the 550delA mutation, while the Y537X mutation was found only in 1 family. We report the results of the screening of CANP3 gene on 27 families, 8 of them never investigated, in which one or both mutations have been identified.
Objective. To determine mutation spectrum of CAPN3 in patients from Croatia.

Patients and Methods. During a 3-year-long project concerning etiology and epidemiology of muscular dystrophies in our country, 37 patients from 27 families with potential calpainopathy were selected by clinical and family study. In the only sporadic patient the diagnosis was confirmed by CAPN3 Western blot. Beside the 550delA mutation in exon 4, two new mutations (R49H and R541W) and one gross deletion (F200-L204del) were identified by DHPLC, Transgenomic Wave System. Furthermore, we developed screening methods for these mutations which included PCR and use of restriction enzymes. Results. Analysis of 54 CANP3 chromosomes by 5 mutations revealed the presence of 550delA in 36/54 (67%), R541W in 3/54 (5.5%), R49H in 1/54 (1.85%), Y537 in 1/54(1.85%) and delFWSAL in 1/54 (1.85%).
Conclusions. CANP3 gene screening by 5 mutations is able to identify 77% of patients with calpainopathy (LGMD2A) in our population. R541W and R49H are novel mutations.

P0513. Lack of association between endothelial nitric oxide synthase gene polymorphism and coronary artery disease in the Greek population

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Genetic polymorphism in the gene for endothelial nitric oxide synthase (ecNOS) has been considered as a potential risk factor for the development of coronary artery disease (CAD) in some populations. We studied a 27 base-pair tandem repeat polymorphism in intron 4 ecNOS gene in 105 control and 82 patients of Greek population. The patient group consisted of subjects aged under 58 years presenting with symptomatic CAD, documented by coronary angiography. The dada between the two groups were analysed by chi- square test. We found no significant difference in the frequency of 4ab genotypes between patients and controls. The frequencies for ecNOS4bb, ecNOS4ab and ecNOS4aa genotypes were 0.69, 0.28, 0.03, respectively, in controls compared to 0.71, 0.24, 0.05 in patients. Thus, in contrast to earlier findings by others from some Asian populations, we have found no evidence for an association between ecNOS4a allele as well as ecNOS4aa genotype and the risk of premature coronary disease in the Greek population.

P0514. Congenital Myasthenic Syndrome in southeastern European Roma (Gypsies)

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Congenital myasthenic syndromes (CMS), a heterogeneous group of disorders arise from various defects of the neuromuscular transmission. The majority of CMS are inherited as autosomal recessive traits due to loss-of-function mutations of the acetylcholin receptor (AChR) subunit genes or the collagenic tail subunit of acetylcholinesterase (ColQ) gene. Many of the e subunit deficiencies, as most frequent causes of CMS, were found to be due to "private" mutations and produce clinical phenotypes with great variations.
This study summarizes data of the genetic analysis of a common mutation, a homozygous basepair deletion in exon 12 of the AChR e subunit gene (e1267delG) in 66 CMS patients from 46 non-related families. All patients were clinically characterized as having sporadic or autosomal recessive CMS. All e1267delG families were of Romani (Gypsy) and/or southeastern European origin. Phenotype analyses revealed a uniform pattern of clinical features including bilateral ptosis, moderate fatigable weakness of ocular, facial, bulbar and limb muscles, positive response to anticholinesterase treatment and a benign natural course of the disease. Genotype analysis was carried out and indicated a common ancestor (founder). We conclude that the mutation e1267delG might be the most frequent cause of CMS in patients of Romani (Gypsy) ethnic origin. Therefore, carrier and/or newborn testing may be beneficial in this group, as the disease is treatable. Moreover, additional haplotype studies in patients harboring e1267delG may enable a more accurate age-dating of this particular mutation. This may help to understand the origin, evolution and dispersion of the disease in the Romani populations.

P0515. Melanocortin 4 receptor mutations - a cause of common obesity

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Background: Human obesity is a prevalent, complex disorder, which is now reaching epidemic proportions in the Western world. It is assumed that genetic disposition to obesity determine susceptibility to environmental factors. Discovery of the molecular basis for obesity in the Agouti mouse has implicated the melanocortin 4 receptor (MC4R) in the development of obesity in rodents as well as in man. **Aim**: The aim of the present study was to analyze the prevalence of *MC4R* mutations in a Danish sample of obese subjects. **Subjects and Methods**: Using PCR-dHPLC and direct sequencing, we screened a cohort of 751 obese male subjects with early-onset obesity recruited at the draft board examinations as having a BMI ≥ 31 kg/m^2 (BMI=33,3±2,4 kg/m^2) and a control group consisting of 706 randomly sampled draftees (BMI=21,4±2,1kg/m^2).
Results: A total of 14 different nucleotide substitutions were identified. Among these, 11 nucleotide substitutions were found only in the obese study cohort. Among these, 9 carriers of a Tyr35Ter variant and 6 carriers of the Arg165Gln variants were identified and showed direct association to obesity (p= 0.004 and p=0.02, respectively). Combined, the carrier prevalence of mutations in the *MC4R* among obese Danish men is 2.8%. **In conclusion**, we find a 2.8% carrier frequency of possible functional variants of the *MC4R* gene among obese Danish subjects which is consistent with reported carrier frequencies in other European populations, making the genetic variability in the *MC4R* the most common cause of genetically determined human obesity with known aetiology.

P0516. Spectrum of Genetic Disorders among Saudi Populations

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The Kingdom of Saudi Arabia occupies over 2/3rd of the Arabian Peninsula The overwhelming majority of the population of Saudi Arabia are Arabs belonging to different nomadic tribes. In early 1960s, investigations began to identify the nature of genetic disorders in the Saudi population and to determine the frequency of the common abnormal genes. Among the single gene disorders, a large number of disorders are identified and some exhibit polymorphism in certain regions of Saudi Arabia. These include sickle cell, a- and b-thalassaemia and glucose-6-phosphate dehydrogenase deficiency genes. A significant number of amino acidurias and other inborn errors of metabolism have been reported. The majority of these disorders contribute significantly to the development of mental retardation in Saudi population. Cystic fibrosis, congenital hypothyroidism, Bardet-Biedl Syndrome, fragile X syndrome, Lower syndrome, and a number of other genetic disorders, have been reported amongst the Saudis. Chromosomal disorders contribute significantly to morbidity and mortality in the Saudi population. Amongst the most frequent Down's syndrome which occurs at a frequency of 1 in 800. Multifactorial disorders are the most common genetic defects and are significant factors underlying morbidity amongst the Saudis. The community studies clarified several of these disorders including diabetes mellitus, hypertension, obesity and cardiovascular diseases. A comprehensive national study has determined the contribution of genetic causes in the development of disability in different regions of Saudi Arabia.
This paper will present an overall coverage of genetic disorders in the Saudi population and suggest preventive measures.

P0517. Genetics diversity of microsatellite loci in populations of Siberia and Central Asia

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1600 individuals from 23 populations belonging to 13 ethnic groups living on the territory Siberia and Central Asia was investigated. These are Northern and Southern Altai, Tuvinian, Buryat, Evenk, Yakut, Kirghize, Uzbek, Tajik, Dungan, Russian, Ukrainian, Tatar. Nine autosomal (D4S397, D5S393, D7S640, D8S514, D9S161, D10S197, D11S1358, D12S364 and D13S173) microsatellites loci (STR) have been studied. Automated genotyping of STR loci was performed with HEX-, TET- or FAM-labeled primers with the ABIPrism310 genetic analyzer and Genescan software. High level of microsatellite loci diversity was shown. In total, 128 different allels at nine studied loci were found. Only 2.5 % of genetic variability of microsatellites was attributable to the differences between populations, whereas 97.5% of genetic diversity was found within populations. Autosomal STR system is most adequately for detection of genetic relationship between closely-related group of populations. The greatest level genetic differentiated of local populations was observed in three Tuva populations (FST=2.68%). Principal component analysis and phylogenetic analysis showed substantial difference between gene pool of Altai and Indo-European populations. High similarity of gene pool in local populations within certain ethnic group was demonstrated both by principal component and phylogenetic analysis. Phylogenetic analysis revealed genetic close relation between Evenks and Buryats.

P0518. Demogenetic study of three populations within a region with strong founder effect

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For more than three decades, the Saguenay population (Quebec, Canada) has been the subject of many studies in population genetics. It has been shown that 82% of the region's gene pool originated from nearly 2600 founders who settled in New-France during the 17th century. Some of these founders are believed to have introduced rare deleterious genes in the Quebec population. The demographic behaviour of these founders' descendants is at the heart of the process by which some of these rare alleles were transmitted and are now found at an elevated frequency in the contemporary Saguenay population. This study aims to a better understanding of the origins and stratification of the Saguenay gene pool. The region has been divided in three sub-populations based on geographical and historical criteria: Lower Saguenay, Upper Saguenay and Lac St. Jean. Three hundred extended genealogies (100 for each sub-population) have been reconstructed, using the BALSAC population register. These genealogies have an average depth of 10 generations. Kinship and inbreeding measurements, as well as the founders' genetic contribution, frequency and number of occurrences were examined. Founders' geographical origins, intraregional migratory movements as well as frequency and concentration of surnames were also considered. Preliminary results indicate lower inbreeding coefficients within the Upper Saguenay population, which is more industrialized and has benefited from a dynamic migratory input. Kinship coefficients between the three sub-populations become distinguishable from the 6th generation onward; highest values are those between Lower Saguenay and Upper Saguenay.

P0519. Polymorphisms of G894T, C774T and VNTR NOS3 gene in the Siberian Populations.

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The study of three polymorphism's (G894T, C774T and VNTR) of endothelial nitric oxide syntase gene (NOS3) in Siberian populations of different race and ethnic specificity (Russians, Tuvinians, Buryats, Yakuts) was carried out. For the studied markers the distributions of genotypes in the most populations corresponded to Hardy-Weinberg equilibrium (HWE). A deviation from HWE was shown only for C774T in Russian and Tuvinians ($p<0.01$). There were an excess of allele C774 (82%) in Russian and G894 (69%) and VNTR B (93%) in Buryats. Have been shown significant difference in alleles prevalence for all markers between Russian and Buryats and for VNTR between Russian and Tuvinians.These data append accumulating information on Siberian populations gene-poll; and there are all reasons to deem that the studies in this field will help to resolve both fundamental biological issues, and appliedones, medical, that is especially actual in the light of genomic medicine development.

P0520. Low density lipoprotein receptor gene mutations and polymorphisms in St.-Petersburg patients with familial hypercholesterolemia.

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Familal hypercholesterolemia (FH) is one of the most common inherited diseases (1:500), leading to atherosclerosis and premature myocardial infarctions. FH is caused by mutations in the human low density lipoprotein (LDL) receptor gene. The early diagnosis of disease by means of DNA analysis allows to perform genetic counseling and to prevent the development of the disease in the FH patients by drug treatment. Aiming to identify the FH-causing mutations of the LDL receptor gene we have screened the separate exons of the gene in collection of 42 FH probands by means of PCR/SSCP analysis followed by DNA sequencing. During this study we have identified the following DNA mutations: C74X (c.285 C>A, TGC→TGA), E397X (c.1252 G>T, GAG→TAG), G571E (c.1775 G>A, GGG→GAG), that seems to be causative for FH development, and two neutral mutations: H229H (c.750 T>C, CAT→CAC), T705I (c.2177 C>T, ACC→ACT). All the mutations with the exception of C74X and T705I were new and not reported elsewhere. Besides the mutations we have identified five neutral changes considered as DNA polymorphisms, namely c.66 C/T (C18C, TAC→TAT), c.1171 G/A (A370T, GCC→ACC), c.1773 T/C (N570N, AAT→AAC), c.1959 C/T (V632V, GTC→GTT), c.2231 G/A (R730R, CGG→CGA). We have compared the occurrence of the polymorphism alleles in FH group and in the group of control (68 non-related subjects) that have not differed besides allele C of c.66 C/T polymorphism and allele A of c.1171 G/A polymorphism were more common in control rather than in FH group.

P0521. Impact of Misspecifying Parental Relationships in Maximum Lod Score Affected Sib-Pair Method

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Many linkage studies are done in small isolated populations and populations where marriages between relatives are encouraged. In these populations, missing information on pedigree structure is quite frequent. Here, we study the impact of misspecifying the parental relationships of the sib-pairs in the Maximum Lod Score method (Risch, 1990) and on the triangle constraints (Holmans, 1993). Characterising the parental relationships by the kinship coefficient between the parents (f), the maternal inbreeding coefficient (a_m) and the paternal inbreeding coefficient (a_p), we studied the behaviour of the identity by descent (IBD) vector expected under the null hypothesis of no linkage with respect to these quantities. We find that the expected IBD vector is not anymore (0.25, 0.5, 0.25) when they differ from zero. There is an increased probability of sharing one or two alleles IBD. And in some cases the vector is even outside the triangle constraints.
We simulated data on two different family structures: (1) parents are double first cousins ($f = 0.125$, $a_m = a_p = 0$), (2) each parent is offspring of first cousins ($f = 0$, $a_m = a_p = 0.0625$). We then analysed the data underestimating the parental relationships. We find that ignoring the kinship and/or inbreeding of the parents increases the type I error of the test when data on the parents are not available. But when parents are typed, we observe a decrease in type I error. We also point out some unpleasant feature of the software GENEHUNTER even when parental relationships are properly specified.

P0522. Evaluation of a cluster of congenital abnormalities particularly Down syndrome in a small Hungarian village

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A unique cluster of congenital abnormalities, particularly Down syndrome and twins was detected in a small Hungarian village in 1989-1990.
Of 15 livebirths 11 (73.3 %) were affected by congenital abnormalities and 6 (40 %) were twins. Of eleven malformed babies 4 had Down syndrome.

The usual causes of congenital abnormality clusters : familial inheritence, consanguinity, classical teratogenic factors including alcohol could be excluded.
Different approaches of field studies, including a case-control study, and laboratory examinations indicated the germinal mutagenic and teratogenic effect of the excessive use of trichlorfon at local fish farms.
The content of this chemical was very high in fish (100 mg/kg) and several pregnant women, including all mothers of babies with Down syndrome, had consumed contaminated fish in the critical period for the congenital abnormalities observed.
Here the main experiences of the environmental abuse are summarized.

P0523. Gene-environment interaction in hereditary haemochromatosis: effect of excessive alcohol use on iron overload in patients homozygous for the C282Y mutation

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Hereditary haemochromatosis (HHC) is the most common inherited disorder in Caucasian populations. Characterised by an iron overload, the disease is associated with increased risk of hepatocarcinoma but is effectively treated by phlebotomies. The HFE gene was cloned in 1996 and a single mutation (C282Y) is responsible for 80-95% of cases. HHC presents a genetic heterogeneity and its expression can be modified by environmental factors. The aim of this study was to identify the influence of alcohol use on the intensity of iron overload, measured by serum ferritin, in patients homozygous for the main mutation.
We retrospectively registered patients C282Y/C282Y treated in a blood centre of western Brittany. A clinical questionnaire was completed at their first visit, informing on biological and clinical signs, life customs, …. The analysis was made using a linear regression model.
This study included 351 subjects of whom 60.4% were males.
The most frequent clinical signs were: weakness (58.5%), arthritis (44.7%), melanoderma (28.1%) and hepatomegaly (14.8%). Twenty-five patients had an excessive alcohol use (>60 g/day - 7.2%). Those subjects presented a significantly increased serum ferritin (1865.5 vs 940.3 ng/ml - p<0.001) and a higher risk of hepatomegaly and melanoderma. The result of regression analysis remained unchanged after adjustment on sex and age at onset.
Excessive alcohol use accentuates iron overload in C282Y/C282Y patients. This aggravating factor increases the risk of cancer and of cirrhosis. Consequently, it is recommended to those patients to have a reasonable alcohol use. This study illustrates an interaction between genetic and environmental factors.

P0524. A comprehensive analysis of Arab and Berber maternal lineages in Morocco.

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Moroccan mtDNA sample (N~540) was collected from different localities of the country and an ethnic origin of individuals (~320 Arabs and ~220 Berbers) has been established for the last 100 years.The variability of the sample was studied using mtDNA hypervariable region sequencing and extensive RFLP typing of phylo-genetically informative coding region positions. Sub-Saharan African lineages (L) comprise more than a fifth of the sample, being, however, somewhat more frequent among Arabs than Berbers, though a relative proportion of subtypes of haplogroup L does not differ among the two groups. In both populations, the rest of the lineages is split between 10%-11% African-specific varieties (U6 and M1) and those, typically found in western Eurasia. Meanwhile, Berbers possess haplogroup V at three-fold higher frequency than Arabs (~6.4% and ~2.2%, respectively) while the content of the pre-V variants differs less (~4.6 and 2.8%). Furthermore, Moroccan Afroasiatic speakers differ from northeastern African and Near Eastern Arabs (as well as from Ethiopians) in one more important aspect: while the latter populations possess a significant proportion of a variety of sister branches of H and V, in particular, preHV, such mtDNA variants are much less frequent or nearly absent in Morocco, whereas haplogroup V is virtually absent in northeast Africa and in the Near East. That, and several other phylogeographic arguments suggest a limited recent migration of female lineages alongside the northern Africa and should be considered as an important aspect where the spread of languages and genes is discussed.

P0525. Maternal legacy of Bretons reveals common features with the extant Scottish mtDNA

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Information about the variability of maternal lineages in France is very limited. Here we bring new data about 400 mtDNAs from different localities in France with a specific emphasize to Brittany. We have analyzed about 120 mtDNAs of native northern Bretons by HVR sequencing and extensive search for coding region polymorphisms. Phylogenetic analysis was carried out using median networks.
All found in Bretons mtDNA lineages belong to well-established European-specific haplogroups H, I, J, K, T, U, V and W. French mtDNA samples from different localities were analyzed likewise. The obtained phylogenetic trees were compared with mtDNA variability in English, Scottish, Irish, Welsh, German and Iberian populations, known from published data. This extensive search over more than 3000 mtDNAs revealed that, apart of trivial overlaps among common all over western Europe mtDNA lineages, there is a subset of more rare mtDNA variants present both in Bretons and Scots. It may suggest that a part of maternal inheritance of northern Brittany gene pool may come from Scotland, in accordance with historical data indicating the replacement of the imperial Roman Legions from the northern England to the coastal Brittany some 1500 years ago.

P0526. The phylogeographic context of the southern Slavs: A mitochondrial perspective

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Mitochondrial DNA lineages of three South Slavonic-speaking populations of the northwestern Balkan peninsula - Croats, Bosnians, and Slovenians (N ~1,200; ~370 haplotypes) - were identified combining the sequences of mtDNA HVS-I region and the RFLP data from coding region. These lineages were compared with a dataset of about 12,000 samples from elswhere. This phylogeographic knowledge base was used to interpret demographic events of the past since the peopeling of Europe. An absolute majority of the lineages found belong to the common western-Eurasian haplogroups - H, I, J, K, T, U, V, and W. Low-frequency haplogroups, e.g., N1, R, HV, and pre-HV, are present as well. Lineages, characteristic for sub-Saharan Africa or eastern Eurasia, occurred in single cases.
For better phylogeographic resolution the data of the populations from different geographic areas were compared in a sub-haplogroup level, and the fraction of the identical haplotypes between population groups was determined. The distribution and diversity of many subhaplogroups reveals that the gene pool of the populations of northwestern Balkans has not gained much influence from the Near East during the Holocene. With some interesting exceptions, southern Slavs tend to have more common phylogenetic branches shared with Germanic (e.g. T2), West Slavonic, or, in some cases, with Finno-Ugric speakers (e.g., U4, U5), but significantly less so with southern European and eastern Mediterranean populations.

P0527. Alu-deletion polymorphism at CD4 locus correlates with Mongoloid component in the gene pool of Northern Eurasia population.

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Insertion-deletion Alu-polymorphism at the CD4 locus located on chromosome 12 has been studied in more than 1400 individuals sampled from 11 ethnic groups inhabiting Siberia and Central

Asia. The investigated populations related to two basic racial types of Eurasia: Mongoloid and Caucasoid. In contrast to other polymorphic Alu-insertion the presence repeat (allele Alu+) is ancestral for insertion at the CD4 locus. Primates (chimpanzees, gibbons and gorillas) are found to be monomorphic relative to insertion presence. All studied ethnic groups are polymorphic at the CD4 locus. It is interesting to note that deletion frequency (allele Alu-) decreases with increased Mongoloid component in the population. Maximal frequency of deletion allele is shown in Russians (0.339). It decreases to the east and is minimal in Yakuts (0.025). For Caucasoids living in Central Asia, i.e. Tajiks and Uzbeks the frequency of Alu allele was 0.143 and 0.130, while those for Mongoloids, i.e. Kazakh and Kirghiz was 0.129 and 0.125, respectively. In the territory of East Siberia the intensity of Mongoloid signs increases in the following series: Tuvinians, Buryats, Dungans, Evenks and Yakuts, while the frequency of Alu-deletion decreases as 0.074; 0.051; 0.045; 0.043 and 0.025, respectively. The study proved the importance of analyzing insertion-deletion polymorphism at the CD4 gene, which is very informative. This polymorphism may probably serve as a marker correlated with the degree of the Mongoloid component in populations. It may be also useful for the specialists of related fields - ethnographers, archaeologists and anthropologists.

P0528. Association between Matrix Metalloproteinase-1 (+2506) gene polymorphism and Early Onset Periodontal Disease (EOP)

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Background: Periodontal disease is a chronic inflammatory disease of the supporting tissues of the teeth, starting with gingivae and progressing to gradual destruction of the bony support and peridontal attachment of the teeth. Early onset periodontal diseases (EOP) are a group of inflammatory disorders characterised by a rapid rate of periodontal tissue destruction, in young individuals. There is now substantial evidence to suggest that genetic factors play a role in the pathogenesis of EOP.
Polymorphisms in the MMP-1 gene, which may underpin inter individual differences in MMP-1 synthesis and secretion have been associated with other diseases, which have an inflammatory pathogenesis. Genetic variation within candidate genes in EOP patients may represent a mechanism by which individuals are rendered susceptible to disease.
Objective: To investigate whether three biallelic polymorphisms occurring within the exonic regions of the MMP-1 gene (positions +85*, +2506** and +2613**) are associated with EOP.
Methods: The MMP-1 polymorphisms were detected using a PCR-SNP-SHOT method. MMP-1 polymorphisms were examined in 72 patients with EOP and 91 healthy matched UK controls.
Results: No differences in allele and genotype frequencies were observed for the +85 and +2613 SNP's. In contrast significant increases in the +2506 A allele and A/G genotype were observed in EOP cases.

	Controls		Cases	
MMP-1 (+2506)	N=91	%	N=72	%
Genotype				
G/G	78	85.7	41	56.9
G/A	11	12.1	29[1]	40.3
A/A	2	2.2	2	2.8
Allele				
G	167	91.8	111	77.1
A	15	8.2	33[2]	22.9
[1]O.R.=4.9, C.I. 95% 2.3-10.6, χ^2 =17.3, P<0.05 [2]O.R.=3.3, C.I. 95% 1.7-6.3, χ^2 =13.8, P<0.05				

Conclusion: Several polymorphisms exist in the MMP-1 gene that influences the MMP-1 biological activity. Our results demonstrated association for EOP risk with the A allele and G/A genotype of the MMP-1 (+2506) gene.
*Exon 1
**Exon 5

P0529. "Implication of population genetics dimension in unrelated bone marrow transplantation organisation"

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The human major histocompatibility complex (MHC) which contains polymorphic multicopy genes such as HLA (Human Leukocyte Antigen), is particularly interesting in terms of polymorphism and genetic markers.
For bone marrow transplantation, most allogeneic transplants are from HLA-identical sibling donors. However many patients do not have a suitable familial donor and transplants from unrelated volunteer donors are required.
Since 1980, Donor Registries have gathered more than 7 x 10^6 potential donors worldwide.
We analysed the 100 000 HLA- A-B-DRB1 typed French donors, and patients since 1998. Haplotype frequencies, regional distribution and comparison between donors and patients HLA distribution, provide good basis for the optimisation of new recruitment strategies. We assume that within the MHC "Polymorphic Frozen Blocks", new SNP inside and outside the genes and Microsatellites would characterise some genetic profiles (including non-HLA genes). Pre-test for such markers would help choosing which entering donors to fully HLA type. Some of 150 highly polymorphic MHC Microsatellites are in linkage disequilibrium with HLA alleles. We tested the ability of 6 Msat to predict HLA types on 800 DNA. To investigate differences and similarities compared to SNP, we reanalysed HLA class 1 alignment. These technical and genetic issues associated with economical considerations contribute to imagine new scenarios for current BMT organisation where the most polymorphic human system is playing a central clinical role. [Work performed as part of an EU contract QLG7-CT-2001-00065: MADO].

P0530. The effect of DNA polymorphisms in angiotensin I-converting enzyme and angiotensin II type1 receptor genes with arterial blood pressure levels in Serbian population

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Polymorphism of angiotensin I-converting enzyme gene (ACE) and angiotensin II receptor gene (ATR1), the crucial components of the renin-angiotensin system, have been investigated with respect to regulation of arterial blood pressure. **Aim of the study:** Possible association between polymorphisms of the I/D in the ACE gene, and the A1166C of the ATR1 gene and arterial blood pressure and serum lipid levels in human population from the Belgrade area.**Subjects and Methods:** We have investigated 285 healthy persons recruited from the general population. Genotyping was performed by polymerase chain reaction (PCR) using a modified tree primer method for I/D and genotyping of A1166C by using allele specific amplification.
Results: Frequencies of the genotypes were a) for ACE - 0,18 (II), 0,51 (ID), 0,31 (DD), with allele frequencies 0,43 (*I*), 0,57 (*D*); b) for ATR1 - 0,54 (AA), 0,38 (AC), 0,08 (CC) with allele frequencies 0,73 (*A*), 0,27 (*C*). In males there is a correlation of the DD genotype with hypertension (OR=4,25; p=0,05). Frequencies of genotypes and alleles of A1166C significantly differ between the hypertensive males and controls (p<0,05). The male persons with CC genotype have an three time higher relative chance of being hypertensive. In two-way ANOVA, a synergistic effect of ACE and ATR1 on the total cholesterol and LDL cholesterol was obtained. The male persons with DD/CC genotype have an eight time higher relative chance of being hypertensive. **Conclusion:** The results of this study are a contribution to prevention and therapy of hypertension and cardiovascular disease in our population.

P0531. Mutation analysis of PAH gene in phenylketonuria patients from Moscow region

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Phenilketonuria (PKU), one of the common inborn error of amino acid metabolism, is an autosomal recessive disease caused by mutations in phenilalanin hydroxylase gene (PAH). Up to date it is known more than 400 mutations but in every population there are some most frequent for each other. We have create the multiplex system for

ACRS PCR analysis for most prevalent mutations in Europe (R408W; R158Q; P281L; I65T; IVS10-11g→a; IVS12+1g→a; R261Q; R252W) and carried out the mutation analysis in PKU patients from Moscow region. Among 94 alleles studied 47 (50%) were positive for R408W mutation, 8 (8.7%) for IVS10-11g→a mutation, 6 (6.3%) for P281L mutation, 2 (2.2%) for R252W mutation and 1 (1%) for IVS12+1g→a. No allele was positive for R158Q, I65T and R261Q mutations. The total informative of our system was 68% for our patients. The informative of this system were 87.4% for St-Petersburg PKU patients and 80.5% for Novosibirsk PKU patients (by published date). The R158Q and R261Q substitutions were detected in this regions but the frequent of Mediterranean origin mutation IVS10-11g→a were less (0.7% and 1.5% correspondently). All alleles with R408W mutation for which it was possible to determined the gaplotype (n=10) had the same VNTR3/STR234 minigaplotype. We propose that the population from central Russia include Moscow region has the other frequent mutation(s) differ from other and more sensitive technique for its detection has to be used.

P0532. Phylogeography of maternal and paternal DNA lineages in South Siberia

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Based on mitochondrial DNA and Y-chromosome variability data the genetic structure of seven Southern Siberian groups, Tuvinians, Todjins, Burjats, Sojots, Tofalars, Altaians and Khakassians (the total sample size is 480 individuals) was described. It was shown that populations studied were formed on heterogeneous genetic substratum encompassing both Asian and West-Eurasian components. Phylogeographic analysis of Europeoid lineages specific for Siberian populations confirms the “southern” origin for the majority (60%) of mtDNA lineages and assumes that their occurrence is due to migrations of Europeoids from West Asia. It was shown that the Y-chromosome lineages of HG10 as well as mtDNA lineages of five (A, B, C, D, X) haplogroups specific for Native Americans are present in Altai and Sayan populations gene pools. This fact together with the presence of palaeo-Europeoid component, represented by Y-chromosome 92R7T-lineages, in Altai and Sayan populations gives an evidence of the participation of South Siberian aboriginal groups in peopling of Americas. The high frequencies of Y-chromosome HG12, which is ancestral to HG16, in populations of Altai and Sayan region (up to 30% in Tofalars) were revealed thus indicating the autochthonous Siberian origin of Y-chromosome TAT-C lineages. The results of phylogenetic analyses based on mtDNA and Y-chromosome variability data testify the considerable inter-population differentiation of Southern and Eastern Siberian groups studied. The degree of genetic differentiation is defined rather by geographical, historical and evolutionary factors, than linguistical and anthropological ones. This work was supported by RFBR (99-06-80430) and the Ludwik Rydygier Medical University in Bydgoszcz (BW66/2002).

P0533. Nonsyndromic hereditary hearing loss in Tunisia: Molecular study and impact of consanguinity

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In industrial countries, about 60% of congenital hearing impairment cases are due to genetics defects and about 80% of hereditary hearing loss are nonsyndromic recessive deafness. Although more than 30 recessive genes have been localised, mutations involving the Cx26 gene (DFNB1) are the most common cause of deafness in many populations. In order to determine the genetic basis and the impact of consanguinity on the hereditary nonsyndromic hearing impairment in Tunisia, we have conducted an epidemiological and molecular study using 117 families with at least two affected children and 236 healthy individuals. These families were ascertained from deaf schools from the whole of Tunisia. Clinical investigation was done on all affected and unaffected individuals. Linkage analysis was undertaken in informative families and sequencing was done when families were found to be linked to one DFNB known gene. In all families and unrelated controls, Cx26 mutations were screened by DGGE and/or sequencing. Our results indicated that all families present a recessive mode of inheritance and at least 8 different DFNB genes are involved. Homozygous mutations in the Cx26 gene was revealed in 21 (18.6%) unrelated families. The most frequent mutation found was the 35delG in patients (86.5%) and in the healthy population (1.3%). On the basis of the known apparent mean consanguineous coefficient, we have determined the increase rate (3.40) of recessive deafness due to the 35delG mutation. In conclusion, the consanguinity increases the frequency of recessive deafness gene but didn’t alter the genetic heterogeneity in Tunisia.

P0534. The presence of the western Eurasian mtDNA haplogroup U5 in sub-Saharan Africans

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We have studied mtDNA variation in 370 people from Guinea-Bissau from different ethnic groups. More than 90% of the mtDNAs belong to the sub-Saharan African haplogroups within L1, L2 and L3. Haplogroups M1 and U6 - the two African subsets of macrohaplogroups M and U, respectively - are present at much lower frequencies and exhibit little diversity. Surprisingly, the typically western Eurasian haplogroup U5 was found at a frequency of ~3%. All of these U5 mtDNAs belong to individuals from the Fulbe subpopulation of our sample - people who inhabit sub-Saharan Africa from Sudan to Senegal. U5 appears to be among the earliest branches of mtDNA outside Africa and can be found all over western Eurasia. Since U5 is either absent or extremely rare in Sudan, Ethiopia and Egypt and rare in the Near East, it seems that its presence in the Fulbe of Guinea-Bissau is best interpreted as a result of gene flow from north-western Africa, where U5 is present both among Arabs and Berbers. In contrast, our sample lacks the most frequent west Eurasian lineage clusters, e.g. H, J and T, suggesting that a specific founder event and not recurrent maternal gene flow was operable here.

P0535. ALU Genetic Diversity in British and Indian Populations.

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ALU polymorphisms provide a useful tool to population geneticists for understanding the population dynamics that have occurred over time. We report here a study of Six Alu insertion loci (TPA25, D1, APO, PV92, FXIIIB and ACE) from 20 endogamous caste and tribal populations of India and 5 regionally subdivided populations of Britain. Overall spectrum of variation in these populations is very interesting at different geographical and cultural levels. High level of insertion frequencies was observed in some highly inbred groups. Average levels of heterozygosities were found to be relatively high in these populations (range 41% to 49.8%). The genetic diversity coefficient GST among this group of populations was observed to be high. Phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Comparisons are made with other world populations to understand genetic diversity and dynamics of Alu variation in British and Indian populations.

P0536. Population genetic analysis of CAG repeats of IT15 in populations of Volga-Ural region of Russia

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The mutation causing Huntington disease (HD) is expansion of a trinucleotide repeat in the 5' end of IT15 gene on 4p16.3 beyond the normal range of 35 repeats. The investigation addresses genetic factors associated with normal variation of the CAG repeat polymorphism in HD gene. In the study blood samples of 602 individuals from 8 populations from Volga-Ural region were investigated: 4 ethnic groups of Bashkirs, Tatars, Russians, Chuvashes, Mordvinians, Maris, Udmurts, and Komis. The method involved PCR amplification using primers flanking only CAG polymorphic cite (without CCG repeats) with subsequent PAGE and dyeing with ethidium bromide. In all populations normal distribution of CAG repeats was found (p<0,001) showing unimodal nature peaking around repeat CAG17; the distribution skewness varied from 0.74 in Chuvashs to 1.98 in Bashkirs. 26 alleles containing from 9 to 36 repeats were revealed in total sample. A new sporadic case of HD was revealed in a person clinically silent and without positive family history of the disease with genotype CAG17/36. Fst, Fis, Fit, Gst, Dst were calculated. Four populations showed rather high rate of intermediate alleles (CAG29-35): Russians, Chuvashes, Mordvinians, and Udmurts (0.06, 0.01, 0.02, and 0.01 subsequently). We found extremely heterogeneity of investigated populations as in comparison with each other so with others world populations (Caucasians, Asians, and African Blacks). Total observed and expected heterozigosity for the locus were 0.751 and 0.769 respectively. Almost all populations were in HW equilibrium.

P0537. The impact of prevention strategies on the prevalence of neural tube defects in Hungary, 1987-1999

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Neural tube defects (NTD) are one of the most common and serious congenital anomalies. As a result of prevention strategies, a decrease in prevalence at birth has been reported worldwide. We aimed to investigate the impact of prenatal diagnosis and folic acid supplementation on the live birth prevalence of NTD in Hungary. We included all liveborn, stillborn, and terminated cases diagnosed with NTD and reported to the Hungarian Congenital Abnormality Registry from 1987 through 1999. Additional cases with NTD obtained from our nationwide field study were also recorded. There were a total of 1,772 cases with NTD among 1,497,515 births. Thus, the overall prevalence was 11.8 per 10,000 births. Anencephaly accounted for 25.3%, spina bifida 61.0%, encephalocele 13.7% of all cases with NTD. The proportion of NTD-pregnancies terminated increased from 17% during 1987-1996 to 72% during 1997-1999, while the proportion of live births significantly declined from 58% to 21%. This two-fold reduction in live birth prevalence is resulted from improvements in the accuracy of prenatal detection of NTD-affected pregnancies with an increase in termination of these pregnancies. Previous surveys of mothers showed that most of them took folic acid supplementation during pregnancy but only about 10% had started it before conception. Thus, the impact of primary prevention on the prevalence of NTD is far from optimal compared to the impact of prenatal diagnosis in Hungary in recent years.

P0538. A Reccurent Deletion In Nemo Gene In Slovene Patients With Incontinentia Pigmenti

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Incontinentia pigmenti (IP) (OMIM 308300) is a rare genodermatosis that segregates as an X-linked dominant disorder and is in male almost always lethal prenatally. The disorder demonstrates complete penetrance, but its phenotypic expression is highly variable. In affected females it causes abnormalities of the skin, nails, teeth, hair, eyes and central nervous system. The gene responsible for IP has recently been identified as the NEMO (NF-kB Essential Modulator) gene. A common deletion mutation that removes exons 4 through 10 was observed in approximately 80% of IP patients. Ten Slovene families (14 female patients containing 10 probands) were included in this study. Polymerase chain reaction (PCR) was used for the deletion analysis of the NEMO gene. Three probands showed deletion of NEMO gene. In our study we identified less patients with deletion than expected. In the patients with the detected deletion the clinical diagnosis is thus confirmed and prenatal diagnosis can be made.

P0539. Genetic polimorphism of CYP2C9 and CYP2D6 in the Croatian population

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Genetic polymorphism of drug metabolizing enzymes can lead to severe toxicity or therapeutic failure of pharmacotherapy. Additionally, genetically determined differences in the activity of the metabolic enzymes can increase an individual's susceptibility to certain types of chemically induced cancers and possibly other diseases. Cytochrome P450 (CYP) is one of the most important metabolic systems of the organism involved in the oxidation of different drugs and other xenobiotics.
Based on the metabolic activity the population is divided into three phenotypes: ultraextensive, extensive and poor metabolizers. Inter ethnic differences in the activity of CYP's have been reported. Cytochromes CYP2C9 and CYP2D6 are involved in metabolism of more than 50 drugs. It is questionable which of the mutant alleles should routinely be identified to allow a sufficiently reliable but still practicable estimation of a persons metabolic capacity.
In our study, we inwestigated the prevalence of most frequent alleles of CYP2C9 and CYP2D6 among 200 unrelated Croatian individuals. The most frequent CYP2C9 alleles are 2C9*1 (0.74), 2C9*2 (0.165) and 2C9*3 (0.095).
The prevalence of CYP2D6 alleles was: 2D6*1 (0.863), 2D6*2 (0.040), 2D6*3(0.011), 2D6*4 (0.068), 2D6*5 (0.008), 2D6*6 (0.010).
Pharmacogenetic studies may be of great relevance in clinical practice. It can predict the drug efficiency in patients prior to treatment, help physicians to identify patients susceptible to the development of harmful side effects to specific drugs and, thus, increase safety and efficacy of therapeutic treatments.

P0540. Candidate Gene Polymorphisms In Thrombotic Disease

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Venous thromboembolism (VTE) is a multifactorial disease that depends on variable combinations of acquired and genetic risk factors. Well established risk factors include advancing age, prolonged bed rest and surgery. Genetic risk factors are also common and may play a role in approximately 25% of the individuals who develop VTE. Factor V, Factor II, MTHFR and CBS polymorphisms are genetic markers associated with VTE; their single contribution could be not so evident to allow its identification. It has been postulated that more than one genetic risk factor may co-segregate with a consequent cumulative or synergistic effect on thrombotic risk (1).
A multilocus assay was used to genotype 65 biallelic polymorphisms or mutations within 36 genes (2) in an Italian population (638 individuals) affected (323) or not affected (315) by venous thrombotic events. These genes are involved in lipid metabolism, homocysteine metabolism, blood viscosity, platelet aggregation, leukocyte adhesion and renin-angiotensin system.
Genotype frequencies for all the markers were compared between the two groups. For each locus the association between genotype and VTE event has been evaluated by means of a logistic model, assuming that event's risk depends on the genotype.
Markers showing an association with VTE, witha p-value <0.05 at univariate level, are reported.

Marker	likelihood ratio test p-value
Factor II.G20210A	0.004
Factor V.arg506gln	<0.001
ICAM.gly214arg	0.039
Angiotensin Receptor 1.A1166C	0.047

1) Seligsohn U and Lubetsky. N Engl J Med. 2001; 344: 1222.2) Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, et al. Genome Research. 1999;

P0541. The influence of FV-Leiden, FII-20210G→A and 677C→T MTHFR gene mutations on prenatal development, risk of deep venous thrombosis and ischemic stroke in Czech children / adult patients.

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The aim of this study was to ascertain population frequency of FV-Leiden (FV-L), FII 20210G→A (FII) and of 677C→T MTHFR gene mutations in Czech controls and to assess their impact on pre- / postnatal development due to hemocoagulation disturbances. In controls FV-L was found in 25/436 (5.7 %), FII in 3/130 (2.3 %) and MTHFR in 74/198 (37.4 %). In the case of deep venous thrombosis in children / adults only FV-L was significantly increased to 8/44 (18.0 %) / 25/160 (15.6 %; p<0.001). In ischemic stroke and transient ischemic attacks in children / adults there was a tendency towards an increase of FV-L 3/22 (13.6 %) / 7/50 (14.0 %; p<0.08). Interestingly, FV-L was found to be associated with an increased risk of stillbirth 4/24 (16.6 %; p<0.05). In a cohort of females with reproductive disorders, referred for genetic testing from a collaborating assisted reproduction clinic, there was a trend towards a decrease of FV-L 4/134 (3.0 %; p<0.08) in agreement with recent hypothesis (Lancet 358: 1238; 2001) on improved embryonic implantation in FV-L carriers. No differences were observed in females with recurrent abortions (I.-II. trimester) in all tested thrombophilic alleles. However, FII and MTHFR mutations were not different from controls in all studied diseases, while verification of observed trends requires further analysis.
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P0542. Y-Chromosome biallelic polymorphisms in Afro-Brazilian populations

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The Brazilian population originated by the admixture of European colonists, mainly Portuguese, African slaves and autochthonous Amerindians. To evaluate the degree of admixture and genetic diversity in Afro-Brazilians, we analyzed nine biallelic markers (M34, PN2, SRY2627, SRY8299, SRY1532, 92R7, DYS271, DYS199, DYS287) in 209 unrelated men, being: 39 from two isolated communities; 88 urban Afro-Brazilians, 32 Japanese and 50 whites. We observed that the majority of Afro-Brazilians Y chromosomes are of African origin: 41-77% in the isolated communities and 49-61% in the urban Afro-Brazilians. The frequency of European markers was 9-29% for the isolated communities and 20-34% for the urban Afro-Brazilians.The DYS199T allele, characteristic of Amerindians, was observed in only 6% of the urban Afro-Brazilians of the city of Salvador. The Alu insertion was present in 59 to 75% of the Afro-Brazilians, in 23% of the Japanese and in 18% of the whites. These results show a differential male and female non-African contribution to Afro-Brazilian populations: European men had a larger contribution as compared to Amerindians concerning Y-chromosome, whereas the data from mitochondrial DNA show a larger contribution of Amerindian women to the non-African heritage of Afro-Brazilians. Historical and social data explain these results, that indicate that the Afro-Brazilian communities were formed basically by people of African origin and the non-Africans incorporated were predominantly European men and Amerindian women.
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P 10. Genetic Services, Genetic Screening, and Public Policy

P0543. Hemophilia situation in northwest of Iran

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Despite molecular advances in carrier detection and prenatal diagnosis, in many rural and border area of Iran and Turkey, hemophilia is still a major problem. RFLP/PCR method for factor VIII mutations screening were carried out. Questionnaires for all patients and their families who are receiving clothing factor were obtained. The necessary DNA was gathered using peripheral blood and Protinase K method. PCR and RFLP analysis were carried out according to the literature. Interestingly in many of the villages still it is possible to see affected female with hemophilia A. Even in some families more than 5 members are affected. This happening mostly because of the consanguineous marriage. In more than 70% of the cases the carrier females are marrying with theirs affected first cousin. Therefore their entire child is affected. Despite prenatal diagnosis facilities in the region, still they are not seeking any of them. In more than 60% of the cases family has noted to the disease by accident such as dental operation or general surgery. This makes the duty of the Cytogenesis and counseling centers much heavier. Even it gives an idea to health care organizers that if they going to offer any help in establishing any center in developing countries check many factors like cultural, educational and traditional background of needed people. In this study we are going to present the results of our study in carrier detection as well as sharing our experience with other collogues for working in rural area.

P0544. Polymorphisms Of The Detoxification System Genes, Predisposing To Atopic Asthma

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The metabolism of exogenous substances (xenobiotics) via Phase I detoxification enzymes (cytochromes P450) and Phase II detoxification enzymes (glutathione S-transferases; N-acetyltransferases) demonstrates substantial individual variability, thus predisposing to different multifactorial diseases, such as atopy and asthma. Polymorphism analysis of CYP1A1, GSTM1, GSTT1, GSTP1 and NAT2 genes in 109 asthmatic patients and 90 control individuals from the Northern-Western Russia was carried out. Individuals with GSTM10/0, GSTT10/0 genotypes were at approximately 8,5-fold higher risk of developing asthma (OR=8,50; 95%CI: 3,623-10,956). Proportion of GSTM10/0, GSTT10/0, GSTP1 A/A individuals appeared to be significantly increased in the group of asthmatics (19,3%) than in the controls (4,4%, p=0.0007), with an OR of 5,13 (95%CI: 1,849-14,239). The frequency of the Ile-Val polymorphism of CYP1A1 gene and the most common NAT2 polymorphisms was similar in the control group and in asthmatic patients. Null-genotypes of both genes - GSTM1 and GSTT1, combined with GSTP1 A/A genotype might be suggested as genetic factors predisposing to atopic asthma.

P0545. The Concept of Telegentics in India: A Survey Among Clinicians

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Telemedicine is an umbrella term that encompasses any medical

activities involving an element of distance. It is described as the next frontier in the delivery of health care. Videoconferences and databases facilities are effectively utilized for monitoring public health activities. Telemedicine is creating new way for patients and clinicians to interact and access specialist services which are not locally available. Developing countries have acute shortage of specialist and their services are concentrated in major cities, so in such circumstances, telemedicine can play a crucially role in bridging thegap. A survey was conducted to access the concept of Telemedicine and Telegenetics counseling via Internet among 20 clinicians attending a genetic counseling course, conducted in our department. Participants were from various places of India and most of them were either pediatricians (60%) or gynecologists from peripheral hospitals. A questionnaire was designed to know the general awareness regarding Telemedicine and Telegenetics i.e. the idea of genetic counseling through Internet in India. Cent-percent of the participants agreed that Internet could play a major role in genetic counseling and patient's management at the peripheral level. Most of clinicians thought that Telegenetics can provide immediate accessibility to experts and will greatly enhance the scope of genetic services in our country. More than 95% of the clinicians agreed to join Tele-consultant group and emphasized the idea of making an interactive websites so that they could be actively connected via net with the tertiary care hospitals after they complete the genetic counseling course.

P0546. A Reliable Quantitative Method For Rapid Detection Of Serum Phenylalanine: Application in PKU screening

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Phenylketonuria (PKU) is an inherited metabolic disease, which is characterized by increased level of serum phenylalanine (Phe). The quantitative measurement of Phe in the patient's serum is necessary to confirm the disease, and to distinguish PKU from other forms of hyperphenylalaninemia. In this study, we report a novel method for quantitative measurement of Phe in serum. The method was developed using Proteus Rettgeri bacteria, which produce phenylalanine deaminase (transaminase). These bacteria could convert Phe into phenylpyrovate in the culture medium, which can be easily detected by spectrophotometer using ferric chloride reagent. In this method, the standard curve for Phe ranging from 2-30 mg/dL was linear. This method was applied to 33 PKU samples (serum and blood spots collected on filter paper), which their serum Phe had already been tested using the Gutheri bacterial inhibition assay (GBIA) and the HPLC method. The results were essentially similar. The advantage of this method over GBIA is its ability to measure the serum Phe quantitatively without the use of expensive beta-(2-thienyl)-DL-Alanine. This method is now used in our PKU screening program in parallel with the GBIA and HPLC test. So far, no false positive result has been seen using this method, which is occasionally happens with the GBIA. This method provides a fast, simple, cost effective and quantitative assay for PKU screening which is amenable to automation.

P0547. Ethical,psychological and social aspects of prenatal diagnostics

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Conteporary state of genetic counselling in Czech Republic is on the relatively good level.We have sufficient network of genetic centres (2/1 000 000 inhabitants), number of clinical geneticists -physicians (approx.6/1 000 000) and other graduated in clinical geneticists(8/1 000 000).The level of prenatal diagnoses is on conveniant level: biochemical screening -Triple Test, age indication (35 years), ultrasonic examination (3 times during pregnancy, at least).Due to this secondary prevention 60% of children with inborn chromosomal aberrations are not born.Molecular genetics is quickly developing the number of possible diagnoses is growing.Nevertheless prenatal diagnoses open many ethical, psychological and social problems: The most problematic are the simplified information about pathologic results of biochemical screening, about pathologic karyotype of the fetus (describtion of possible clinical signs of the future child), about possible termination of pathologic pregnancy, about uninformative family with genetic disease caused by rare unusual mutation of well known gene, information about balanced chromosomal translocation of one of the parents etc.Even more tactful information should be to parents who have pregnancy after IVF, waiting a long time for the future child.Even more difficult is refusing of women, who offers donnation of ovum and who for instance balanced translocation or genetic unfavorable family history.The genetic counselling should be everytimes empathetic, fully informative and non- authoritative.

P0548. Pronto Ethnix™ - a Population-Based Approach for Genetic Testing

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Genetic diseases are unique in that their prevalence varies significantly in different ethnic groups or geographical regions. When designing genetic diagnostic kits, we apply an approach that takes into account these unique properties, to increase detection rate and decrease cost.
Our first Ethnix™ panel is aimed towards Jews of Eastern European origin - the Ashkenazim, which are unique in terms of demographic history and genetic structure. Emerging from a limited number of founders, they have undergone an outstanding expansion of population size and have had the tendency to marry within the religion. As a result, Ashkenazi Jews share a very similar genetic background. This similarity includes some recessive mutations, which, when homozygous, lead to progressive deterioration of one's health, life-long disability and death. Relatively few mutations account for most of these diseases.
Our single nucleotide primer extension technology - Pronto™, provides a user-friendly, rapid and accurate method for mutation detection. Using Pronto™, we developed a testing panel for the most common disease-associated SNPs in Ashkenazim. It tests for mutations which cause Tay-Sachs, CF, Canavan, Gaucher, Fanconi anemia, Bloom syndrome, Familial Dysautonomia, Neimann-Pick, Mucolipidosis type IV and Glycogen Storage disease type I. We apply the same approach in our cancer-predisposition panel for Ashkenazim, which tests for the few cancer-causing mutations in Brca1, Brca2 and APC that are prevalent in this population.
Another panel targets the CFTR gene with mutations that are common throughout Europe as well as mutations that are specific for particular regions.
Future Ethnix™ panels will target additional populations.

P0549. Extremely Low Prevalence of Factor V Leiden, FII20210A and FXIIIV34L in Chinese Population

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The Factor V Leiden (FVL) and prothrombin G20210A (FIIG20210A) are the two most commonly recognized risk factors for venous thrombosis. In contrast, a gene variant of factor XIII, FXIIIV34L, has been reported to confer protection against arterial thrombosis. The prevalence of these three variants has been determined for most Caucasian populations, however, distribution of these variants in other populations has been poorly studied, especially in Asian populations. To determine the prevalence the three variants in the Chinese population we analyzed 500 unrelated and healthy women from Taiwan area of China. The carrier frequencies, allele frequencies, and the frequencies for all possible genotypes of FVL, FIIG20210A and FXIIIV34L from these samples are given in the Table. The corresponding data for 500 healthy and unrelated individuals from the Newfoundland Caucasian population is included for comparison (Our unpublished data). Our results show that Chinese population has a 9-fold lower prevalence of FIIG20210A, a 24-fold lower prevalence of FVL and more than 250-fold lower prevalence of FXIII34L compared with our Caucasians controls. The present study demonstrates a significant and highly ethnic-dependent distribution of the three variants in the Chinese population compared with Caucasians. The dramatically lower prevalence of FVL and FIIG20210A in the Chinese population suggests that these two gene variants play significantly less role as genetic predisposing factors

in Chinese with venous thrombosis. Furthermore, the dramatically lower prevalence of FXIIIV34L in Chinese also suggests that this gene variant might not be a useful candidate allele for further study of arterial thrombosis in Chinese.

	Genotype	Chinese (n = 500)	Newfoundlander (n = 500)
	V/V	499 (99.8%)	259 (51.8%)
FXIIIV34L	V/L	1 (0.2%)	209 (41.8%)
	L/L	0 (0%)	32 (6.4%)
Carrier Frequency		0.2%	48.2%
Allele Frequency of 34L		0.1%	27.3%
	G/G	499 (99.8%)	494 (98.8%)
FIIG20210A	G/A	1 (0.2%)	6 (1.2%)
	A/A	0 (0%)	0 (0%)
Carrier Frequency		0.2%	1.2%
Allele Frequency of 20210A		0.1%	0.6%
	W/W	499 (99.8%)	476 (95.2%)
FVL	W/L	1 (0.2%)	24 (4.8%)
	L/L	0 (0%)	0 (0%)
Carrier Frequency		0.2%	4.8%
Allele Frequency of FVL		0.1%	2.4%

P0550. Our first experiences with molecular genetic diagnosis of malignant hyperthermia in the Czech Republic

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Malignant hyperthermia (MH) is an autosomal dominant, potentially letal pharmacogenetic predisposition which is considered to be of the main causes of death during anesthesia. Triggering by volatile anesthetics and depolarizing muscle relaxans in susceptible patients leads to an abnormally high release of intracellular Ca^{2+} in sceletal muscle. In more then 50% of the affected families, MH is caused by mutations in the ryanodine receptor of sceletal muscle(RYR1) encoded by gene on chromosome 19q12.-13.2. Mutation analysis in human RYR1 gene has shown until now about 25 mutations which could be associated with MH predisposition.

To date, MH diagnosis has not been provided in the Czech Republic. We have just started the mutation analysis in RYR1 gene to those patients who either had a documented hyperthermic crisis or positive *in vitro* muscle contracture test (IVCT). The most aim our programe is to discover MH families members at risk. This work is supported by grant from the Internal Grant Agency of Ministry of Health in the Czech Republic (IGA MZ ND 6865-3)

P0551. The pilot genetic testing program in Ukraine

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We have elaborate the pilot genetic testing program based on the information obtained by population screening in different regions of Ukraine. The program involved three categories of populations: 1) members of families with a high risk monogenic hereditary disorders; 2) patients of IVF centers; 3) persons of fertile age from common populations before marriage or before and during pregnancy.

For the first category we have screened the most common mutations and linkage polymorphisms: 320 CF-families, 129 DMD-families, 100 PKU-families and 85 SMA-families. Early prenatal diagnosis was performed based on CVS testing in high-risk families: CF - 66 cases, DMD - 81, PKU - 100, SMA - 85, HD - 2, Fra X - 1, Hemophilia A - 5.

2-nd category. The screening of CFTR gene mutations and long arm chromosome Y microdeletions was performed for 105 infertile man involved in ICSI program. The chromosome Y microdeletions were detected in 5 cases. In two cases we have detected CFTR gene mutation - delF508.

3-rd category. The most common mutations of CFTR, PAH and SMN genes were screened in 175 unrelated persons. 28 persons have been determined as heterozygous carriers of following mutations: delF508 - 5 persons, R408W and Y414C - 2 persons, exon 7 deletion of cenSMN gene - 21 persons. The genetical consulting, partner testing and prenatal diagnosis were recommended for all families at risk. We have suggested that the such genetic testing programs will be useful for prevention of most common hereditary disorders in Ukraine.

P0552. Mutational analyses of potassium channel gene KVLQT1 and identification of a novel long-QT syndrom mutation (T309I)

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Long-QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on ECG and presence of syncope, seizures, and sudden death. Five genes have been implicated in Romano-Ward Syndrome, the autosomal dominant form of LQTS: KVLQT1 (11p15.5), HERG (7q35-36), SCN5A (3p21-24), KCNE1 and KCNE (21q22). Mutations in KVLQT1 and KCNE1 also cause the Jervell and Lang-Nielsen Syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive form.

We used mutational analyses to screen LQTS patients from 23 families for mutations in the KVLQT1 gene, a potasium channel subunits that account for most of RWS cases. In six unrelated LQTS patients, singlestrand conformation polymorphism analyses identified aberrant conformers. DNA sequence analyses identified two missense mutations G325R and T309I, localized in the transmembrane domains S6 and pore region. CT substitution resulting in a tyrosine to an isoleucine transition at codon 309 (T309I) was novel. We also identified two single nucleotide polymorphisms in four unrelated LQTS patients, F484F and Y171Y, which have not been previously reported. Further functional studies will be required to determine what effect each of these changes may have on KVLQT1 channel function.

Genetic screening using mutational analysis can improve presymptomatic diagnosis. Familial and sporadic cases affected by mutations in all LQT genes can now be genetically screened to identify individuals at risk of the development of the disorder. This work is supported by the Internal Grant Agency of the Ministry of the Health in the Czech Republic (IGA MZ 5718-3)

P0553. Periconceptional use of folic acid in The Netherlands: from science to public health policy

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Introduction:

Neural tube defects (NTD) have a multifactorial etiology. Randomized controlled trials have shown that one exogenous factor, folic acid, reduces the risk of NTD with 50-70%. Dutch health authorities advised in 1993 that women who want to become pregnant should take a 0.4-0.5 mg folic acid tablet daily from 4 weeks before conception till 8 weeks thereafter. A mass media campaign in 1995 informed the public of this advice. In the Netherlands 80-90% of pregnancies are planned. The aim of the campaign was to reach at least 70% of women wishing to conceive and that 65% of these women would use it appropriately.

Awareness and use of folic acid:

At first or second antenatal visit, women filled out a questionnaire. The survey was performed 5 times. The percentages that used folic acid are mentioned in the table. In 2000, 26% of low educated vs. 47% of high educated women used folic acid during the entire advised period. Although an impressive proportion of women complies to the advice, the aim (85%*70%*65%=39% appropriate folic acid use) has not been attained. Yet the percentage of users is higher than other figures reported in the literature so far.

Public health policy:

Although fortification of foods is obligatory in some, and admitted in

most countries, the Dutch Health Council advised in 2001 against fortification of regular foods. The Dutch minister of Health stated that the present policy will be continued and attempts to inform women preconception will be intensified.

Percentage of pregnant women that used folic acid		
Year	% any time of advised period	% during entire advised period
1994	7.8	0.4
1995	21	4.8
1996	38	15
1998	62	36
2000	61	36

P0554. Towards a general quality standard in genetic testing

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Genetic diagnostic laboratories show a growing interest in the systematic application of quality assurance (QA) in genetic diagnostic laboratories. Many laboratories now participate in external quality assessment (EQA) schemes. In order to evaluate the status of QA implementation in genetic diagnostic laboratories, a survey on QA and accreditation was sent to 206 laboratories from 32 European countries and 5 laboratories in Australia and the USA.
151 participants responded (73 %) to the survey. In order to minimise errors, an appropriate QA system needs to be worked out for the whole procedure of a genetic analysis. Such system is established in 110 of the surveyed laboratories (73%): 45 laboratories follow an international standard, 52 a local or national standard, and 13 laboratories did not specify which guidelines are being used. However, only 82 laboratories are officially inspected: 52 are inspected by a national body, and 30
are accredited by an internationally recognised accreditation body. Thus, it is clear that at present there is no harmonisation on QA among laboratories. The new ISO 17025 standard, which is accepted by most national and international accreditation bodies, could therefore be an important step towards harmonisation of laboratory practice. This new standard also includes requirements for reporting the results. The reporting results of the latest CF EQA schemes illustrate that less than 15% of the laboratories include all the items required by this new standard.

P0555. Frequency Of Cx26 Mutations In Deaf Newborns Detected Through A Pilot Universal Neonatal Screening In The Piemonte Region

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Deafness in young infants is commoner than cystic fibrosis, galactosaemia or congenital hypothyroidism, affecting one in 1000 children. Early identification of deaf babies and remedial education is beneficial, with better expressive and receptive language, speech, and social and emotional development.
Pilot screening scheme. The primary screening tool is the measurement of transient evoked otoacustic emissions (TEOAE), administered to all newborns before dismissions from the neonatal center. If the TEOAE test is pathological is repeated three times, then the brain-stem auditory evoked response (BAER) will be measured. When deafness is present, the baby is referred to a specialistic center for audiological assessment and for the application of a rehabilitative protocol and molecular test is performed. **Molecular analysis.** In developed countries, deafness has an important genetic origin, and at least 70% of genetic cases are autosomal recessive non-syndromic, 80% of which due to mutations in the GJB2 gene. In this pilot screening genetic counselling is offered to parents of deaf babies. In a previous study with direct sequencing of the GJB2 gene, we found mutations in 43 of 74 cases (58%) of severe/profound deafness. The most common mutation is 35delG, accounting for 74% (30/43) of all deafness alleles. The mutations V95M and E47X were detected four and two times, with a relative frequency of 4.6 and 2.3% respectively. Other mutations (L90P, 290-291insA, 333delAA, W24X, E119del, M34T, Q80P) were detected once. We also found four variants of unknown significance (V37I, F83L, R127H, V153I) and the novel alleles M162V, K224Q and R184Q .

P0556. Prospective study on Genetic Testing Services quality assurance and harmonization in EU

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The use of clinically meaningful genetic tests for humans -for diagnostic, confirmatory and predictive purposes - is expanding in all European countries. A few different national regulatory frameworks already exist but there is no harmonization on a European level to ensure a sufficient level of quality, safety and efficacy of genetic testing services in Europe, which is required by society. Genetic testing services are not covered by Council Regulation (EEC) No 2309/93 laying down Community procedures for the authorization and supervision of medicinal products for human and veterinary use or Directive 98/79/EC on in vitro diagnostic medical devices, which applies only to products to be marketed.
There also seems to be a need to set up a European laboratory network to cover rare diseases. Given that research into genetic mutation is so complex, only a few laboratories are in a position to supply an appropriate test for certain diseases, whereas most European countries have at least one laboratory to deal with the more common diseases. To avoid this difficulty, a network of European laboratories, covering the different diseases and genes, could be set up.
JRC (EC) is currently analyzing the potential need and technical options for harmonization and quality assurance of Genetic Testing Services in the EU, taking in consideration already ongoing activities at European level (EQA scheme for HD, Concerted Action for CF, EMQN, etc).

P0557. Five years experience of biochemical screening in Saint-Petersburg.

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Prenatal Down's syndrome biochemical screening in St.Petersburg is carried out since 1997, as a double test. Total beta-human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) were studied in 123400 pregnancies dated 1997-2001. Proportion of population covered is more than 84 % in 2000-2001 . About 7 % of screened group had a high risk of Down's syndrome (DS) which was calculated by homemade software based on likelyhood ratio and age risk. 74 out of total 191 pregnancies with Down's fetus were screened . Detection rate in women less 35 was 61 %, in advanced age women - 85 %. According to these findings 15-17 weeks of pregnancy are the most informative period for DS testing . Repetitive biochemical testing carried out after 15-17 weeks usually reduces detection rate and make the decision on invasive karyotyping more ambiguous. These initial results also favor
the use of our homemade test -system produced by "Alkor-Bio" (St.Petersburg) supplemented by new software for Down's syndrome screening "MedInformatika" (St.Petersburg).

P0558. Internal Quality Control: Development of new best practice guidelines for diagnostic molecular genetics laboratories.

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Internal quality control (IQC) refers to all of the policies and procedures which a laboratory puts in place to ensure the error-free processing and analysis of all samples. It covers many areas from staff training to documentation of experiments and reporting and is vital to the provision of a high quality molecular genetics diagnostic service.
A best practice meeting on IQC was held in Edinburgh on 3rd April 2001 under the auspices of the European Molecular Genetics Quality Network (EMQN) as a satellite meeting of the UK Clinical Molecular Genetics Society's spring meeting. From discussion at this event a draft set of best practice guidelines has been developed. The general principles of effective IQC (including the associated problems)will be discussed and illustrated with reference to the draft guidelines.

P0559. Screening for heterozygosity of C283Y mutation in Bulgarian Gypsy minority

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Limb-girdle muscular dystrophy type 2C (LGMD2C), a subgroup of sarcoglycanopathies, is caused by mutations in the gamma-sarcoglycan gene, localized on 13q12. Among the described mutations, causing an autosomal recessive muscular dystrophy, a “private” Gipsy mutation C283Y (transition G→A in codon 283) is detected.

The extensive field work in 300 Gypsy living places in Bulgaria revealed about 40 Gypsy patients clinically diagnosed as LGMD. Considering the fact that the Gypsy minority is an isolated population with high percent of consanguinity and having in mind the autosomal recessive type of inheritance of LGMD2C, a raised carrier frequency of C283Y mutation was expected.

Several screenings for determining the percentage of heterozygosity of C283Y mutation among Gypsy minority were performed. The applied method was direct amplification on dry blood spots from Guthrie cards followed by SSCP. Heterozygotes were confirmed by RsaI restriction digestion.

Screening on 400 Gypsy newborns from Northeast Bulgaria showed high percentage of heterozygosity- 2.25%. Screening on 300 volunteers of a reproductive age from Sliven showed very high percentage of heterozygosity - 7.7%. Investigation on 126 volunteers of a reproductive age from Senovo (Northeast Bulgaria) showed that 22 of them were heterozygotes (17.46%). Screening on 50 Gypsy newborns from Stara Zagora region (Middle Bulgaria) showed no heterozygotes.

The above data show that the disease seemed to be geographically localized to Eastern Bulgaria. It is important to construct a precise map of the regions with high carrier and/or disease frequency. Such regions should be with priority in the Bulgarian healthcare system.

P0560. Stable EBV transformed B lymphocyte cell lines derived from residual clinical blood for PE/QA of molecular genetic testing

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Positive control material for performance evaluation and quality assurance (PE/QA) of diagnostic molecular genetic testing (MGT) is difficult to obtain for many genetic diseases. To determine whether control material could be derived from residual blood collected for routine clinical MGT, 51 bloods were collected for EBV transformation. Eleven cell lines representing 5 genetic disorders were successfully cryopreserved. Results from a logistic regression model indicate that sample age and anti-coagulant (ACD or EDTA) were statistically significant predictors of transformation success; age and sex of the patient were not ($p<0.05$; samples collected in ACD and stored for fewer days were more likely to transform). Sample volume, hemolysis, whether or not the tube had been opened and prior storage temperature were included in the model. Successful transformation was achieved in samples up to 13 (EDTA) or 14 (ACD) days old and/or with as little as 1 ml blood from both opened and unopened tubes. Acceptable storage conditions were ambient or 4°C for samples <7 days old and 4°C only for samples 8-14 days old. Average time to transformation was 44.0 ±2.5 (SEM) days for ACD samples and 61.3 ±10.8 (SEM) days for EDTA samples. Stability of mutations was verified in ten cell lines after five 10-fold expansions in culture. Each cell line was sent to 5-6 outside MGT laboratories. All mutations were correctly identified by several methods, indicating that samples of this type are likely to be suitable for use as PE/QA material. (Funded by the Centers for Disease Control and Prevention)

P0561. Genetic screening of Familial Mediterranean Fever mutations in the Greek population

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Familial Mediterranean Fever (FMF) is an autosomal recessive disease that primarily affects populations surrounding the Mediterranean basin e.g. Armenian, Jewish, Arab and Turkish populations. FMF is characterised by recurrent episodes of fever accompanied by abdominal pain, pleuritis and arthritis. The most dangerous complication of FMF is amyloidosis that can lead to end-stage renal failure. The gene for FMF (MEFV) was cloned and missense mutations were found to be responsible for the disease. About 20 mutations have been identified so far, some of them being very freequent. The aim of this study was to investigate the carrier rates of the common MEFV mutations among 250 healthy members of the Greek population. The studied group was consistent only of Greeks whose parents were also of the same ethnicity. Two FMF mutations, V726A and M694V, were considered to be the most common in Greek patients from earlier studies. Our results indicated that none of the studied healthy individuals was carrier of any of these two mutations. We may conclude that the frequency of FMF mutations is extremely low in Greeks. It is possible that the previously identified few FMF Greek patients from others were of different origin.

P0562. DHPLC mutation analysis of Phenylketonuria

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Phenylketonuria (PKU, OMIM S 261600; McKusick 1986) is one of the most common autosomal recessive disorders in Europe and related to mutations in the PAH gene. The frequency of heterozygotes in Europe is 1:50. The basic defect in PKU is phenylalanine hydroxylase deficiency. The disease is characterised by an accumulation of phenylalanine in blood and nervous system, which leeds to mental retardation. A diagnosis early after birth is very important because the disorder is effectibly treatable by an adequat diet. A disease positiv neonatal screening requires molecular investigations to provide a solid basis for genetic councelling. This is particularly important for adult patients with PKU who are planning their own family. This group of individuums is interested in a rapid and reliable molecular diagnosis of their individual mutation status and the exclusion of a heterozygous mutation in their partners.

Denaturing high-performance liquid chromatography (DHPLC) is a fast and sensitive method for mutation screening which has been applied for mutation detection in various disease related genes. This method has not been previously applied to PKU. Therefore we established DHPLC for PKU mutation screening followd by automated sequencing to analyse rapidly the complete coding sequence of the PAH gene in a total of 125 PKU patients from Saxonia and Turkey. We identified 40 different mutations and polymorphisms in a total of 250 PAH-allels. The mutation detection rate with DHPLC was approximatly 98%. DHPLC has proved to be a fast and reliable method for mutation screening in PKU.

P0563. Screening for Cys 282Tyr and His 63Asp mutations of HFE gene in populations of Volga-Ural region of Russia

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Hereditary hemochromatosis (HH) is an autosomal recessive disease affecting iron metabolism commonly found in whites. Populations of northern European origin show the highest frequency of HH, with 1 in 300 individuals affected. Two sites of point mutations in the HFE gene - Cys282Tyr and His63Asp - are associated with greater than 90% of

HH cases. In presence no information is available on the frequency of HH in Volga-Ural region. To define a carrier frequency we screened for Cys282Tyr and His 63Asp mutations of HFE gene in 6 populations of Volga-Ural region of Russia: Udmurts, Mordvins, Tatars, Bashkirs, Chuvashis and Russian from Bashkortostan. The frequency of the Cys282Tyr mutation is highest in Udmurts and Russians - 13,1% and 10,6% respectively, that corresponds to northwestern European populations. In Mordvins, Chuvashis and Tatars heterozygosity for Cys282Tyr is 7,3%, 7,14% and 6,3% respectively and is absent in Bashkirs. Heterozygosity for His63Asp ranges from 17,7% in Tatars to 31,7% in Mordvins and 31,9% in Russians. The His63Asp mutation is less frequent (occurring in 20,8%-24,6%) in Bashkirs, Chuvashis and Udmurts. A small percentage (0%-3,6%) was found to be homozygous for His63Asp and 12,2% in population of Mordvins. We revealed one case of compound heterozigoty for both mutations in Chuvashis population. Taking into account high frequency for heterozygous Cys282Tyr and His63Asp mutations, we propose that frequency of HH in populations of Volga-Ural region corresponds to European populations, so there is need of screening of subgroups at risk to promote early diagnosis and therapy of this disease.

P0564. Genetic counselling for familial fatal insomnia

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Familial fatal insomnia (FFI) is a rare, autosomal dominant progressive prion disease. It is characterised by neuronal degeneration of selected thalamic nuclei and progressive insomnia. Onset ranges from 37-61 years and the average duration of the disease is 13 months. It is associated with a asp178-to-asn mutation of the prion protein gene (PRNP), when the amino acid at position 129 is methionine.

We present the case of a family, in which the father was diagnosed with FFI at the age of 50 years and died 2 years later. Two sons in their second decades attended for genetic counselling, unaware of their father's diagnosis. The wife of one son was 19 weeks pregnant. Issues surrounding possible predictive and prenatal diagnosis were discussed as for other late-onset neuro-degenerative disorders.

We discuss the issues relating to the possible transmissible nature of this prion disease and the obstetric management of our patient, with reference to the public health implications of prion diseases.

P0565. Screening for Down's Syndrome in Estonia 1995-2001.

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Basic statistics. Area: 45214 km2. Population: 1,46 million.

Birth rate in 2000: 13 119 newborns. Maternal age at delivery  35….9.5 % (1227 women). Currently about 7% pregnancies undergo invasive prenatal diagnosis (mostly amniocentesis) in Estonia. Incidence of Down Syndrome (SD) before prenatal screening was started (1990-1994) was 1: 700.

Screening .Chromosome anomalies are screened for advanced maternal age ( 35) since 1995. In 2001 , 48% of women  35 had fetal karyotypes.

During the period when prenatal diagnosis has been used (1995-2001) 29 cases (52%) SD of advanced maternal age risk group have been diagnosed prenatally. During the last three years 72% of the SD cases have been detected prenatally.

Maternal serum screening (double test) is not widely used in Estonia: it is routinely offered since autumn 1998 in Tartu, since 2000 in southern Estonia and since 2001 in Tallinn and other part of Estonia. In 2001, 31% pregnant women in Estonia were monitored. In period of 1998 - 2001 altogether 6500 screening tests were done. Positive serum screening was indication for amniocentesis (fetal karyotyping) in 411 (6,4%) cases. Chromosomal abnormalties were detected in 7 (1:59) cases, SD in 3 cases.

Conclusion. Incidence of Down Syndrome in Estonia since prenatal screening was started in 1995 is 1: 942. In order to reduce the rate even further, a greater percentage of pregnancies in age group  35 have to be monitored by maternal serum screening. In the future first trimester screening is currently under development in Estonia .

P0566. Role of Certified Reference Materials and the Standardisation System in In Vitro Diagnostics

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The In Vitro Diagnostica- Medical Device (IVD-MD) directive (Directive 98/79/EC) requires traceability of calibrators and control materials to reference measurement procedures and/or reference material of higher order. According to the VIM [1] traceability is defined as "property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties". Hence standards reference materials to support the traceability chain and well understood measurement procedures play an important role in achieving traceability.

It is evident that in the field of clinical chemistry complexities of measurands, the biological variability and commutability have to be taken into account. Otherwise the effect of reference methods and materials on standardization will be limited. Nevertheless the IVD-MD directive is a call to improve comparability of measurement results through more structured and understood approaches for standardization.

In this lecture, the traceability chain will be explained and two approaches towards standardisation using a CRM are discussed and will be compared. The certification process including definition of uncertainty values for both, homogeneity and stability as well as the contribution of characterisation will be presented.

[1] International Vocabulary of Basic and General Terms in Metrology, ISO, 1993

P0567. Variations in termination rates in pregnancies diagnosed with Klinefelter syndrome: Data from a cross European study (DADA)

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Introduction: Klinefelter syndrome (KS) occurs approximately in 1 per 800 male live births. 10%-20% are identified by prenatal diagnosis (PD). Most cases are detected incidentially when PD has been performed because of an increased risk for Down syndrome. Because KS is likely to be unfamiliar to the general population, information given about KS may be of crucial importance for parents' decisions about whether or not to continue with the pregnancy.

Methods: A systematic review of the case notes of all KS diagnosed up to 24 weeks of gestation in 8 European regions in 5 European countries (France, Germany, The Netherlands, Spain and the UK) was conducted. The variables documented included maternal age, parity, gestational age at diagnosis, speciality of health professional providing information before and after PD.

Results: Details of 111 pregnancies and their outcome were obtained. Mean maternal age was 36.9 (±4.6) years, 44.1% pregnancies were terminated. Across the 8 European regions termination rates varied between 76.9% and 0%. Using multivariable logistic regression analysis, the only significant predictor of continuation of the pregnancy was the speciality of the health professionals conducting post diagnosis counseling: the affected pregnancy was more likely to continue when post diagnosis counseling involved only a genetics specialist (RR 2.42 (95% CI 1.14 to 5.92)).

Discussion: There is an association between whether or not a woman terminates a pregnancy affected by an unfamiliar fetal anomaly and the professional background of the health professional providing post-diagnostic counseling. The causal nature of this association remains to be determined.

P0568. Reduced Folate Carrier Polymorphism (A80G) and Neural Tube Defects (NTD)

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Impairment of folate metabolism have been observed in families with Neural Tube defects (NTD). The thermolabile 677CT and 1298AC polymorphisms in MTHFR (methylenetetrahydrofolate reductase) gene have been implicated in the pathogenesis of NTDs, but these mutations can explain only in part the protective effect of folate on NTD. Therefore, other defects in folate metabolism such defective carriers could be involved in the ethiology of NTD. Recently, the human folate carrier (RFC-1) gene has been isolated and characterized. A common polymorphism A80G, changing a histidine with an arginine, in the exon 2, has been reported. In this population-based study, we examined the impact of the RFC-1 A80G variant on NTD risk and the potential interaction between this polymorphism and MTHFR A1298C mutation. We report that the RFC-1 A80G variant is common in the Italian population (0.47). Nevertheless, the allelic frequency was higher among NTD cases and their parents. Heterozygous patients and mothers have OR of 1.72 (95% CI 0.96-3.11) and 1.86 (95% CI 0.68-5.27), respectively. More sensitive risk was calculated for the 80GG genotype of cases (OR=2.35; 95% CI 1.21-4.58). On the contrary, the heterozygous genotype of the mothers and both heterozygous and homozygous genotypes of the fathers did not seem to be significant risk factors. According to multifactorial basis of NTDs, we found that combined genotypes for MTHFR A1298C and RFC-1 A80G polymorphisms of cases (1298AC/80GG and 1298CC/80AG) results in greater NTD risk than heterozygosity and homozygosity for RFC-1 A80G variant alone.

P0569. “Screening for PAX6 gene mutations : a five years experience”

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PAX6, a paired box transcriptor factor, is considered as the master gene control for morphogenesis of the eye. Human PAX6 mutations are associated with a range of ocular abnormalities, including aniridia, various anterior segment defects and foveal hypoplasia.
We carried out a mutational analysis of the PAX 6 gene in 54 unrelated patients with aniridia or one of various closely related syndromes. Despite an association of several methods a deleterious variation was evidenced in only 30 patients : twenty four different mutations, 16 of which are novel, were found.
The spectrum of PAX 6 mutations in our population is highly homogenous with 96% of all mutations leading to premature truncation of the protein (8 nonsense and 4 splice site mutations, 11 insertions and deletions) and only one missense mutation (4%). Examination of the phenotype did not allow to recognise significant differences whatever the protein was deprived of one or another of its functionnal domains. We present 22 mutations in association with common recognisable aniridia phenotypes and 2 in association with atypical phenotypes : a missense mutation (R19P) in an individual with an unusual microphtalmia-sclerocornea phenotype and a splice site mutation (IVS4+5G>C) in a family presenting a panocular defect associated with a congenital nystagmus.
Our observations support the concept of dosage effects of the PAX 6 mutations as well as presenting evidence for variable expressivity. Genotype-phenotype correlation in ocular defects related to PAX 6 mutations remains difficult despite the increase of observations.

P0570. Efficiency of the prenatal screening programs in the Czech Republic

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In our republic, biochemical screening for NTD, AWD, Down’s syndrome and trisomy 18 in the 2nd (AFP, hCG, µE3) or the 1st (AFP, PAPP, free ßhCG) trimester of gravidity is available to pregnant women. All of them are examined using specialised ultrasound three times during pregnancy. Women over 37 years are offered fetal karyotyping by cultivation of amniotic or chorionic cells, by FISH or by capillary electrophoresis. Prenatal diagnosis is established in 12 large genetic centres and in several small private clinics. Amniocentesis is done in many gynaecological departments with the experience in fetal medicine. All these investigations are fully paid by Health Insurance. Main principles of our screening policy are: (1) general availability and (2) voluntarity. Biochemical screening applies the following policies: (3) multimarker screening with computer evaluation including nuchal translucency and (4) cut-off for positivity 1:250 – 1:300.
In 2000 nearly 60,000 specialised sonographical investigations (0,6 % efficiency), 8,000 chromosomal investigations (3 % efficiency) and 1,200 DNA analysis (12 % efficiency) were performed, two last investigations in selected patients only. When we compare effectivity of these three prenatal programs for detection of chromosomal aberration, then only maternal age reasons indicated 1.4 % of them, biochemical screening 4.6 % and specialised ultrasonography 11.6 %. Selectivity for Down’s syndrome was 78 % at FP of 5 % and RT 1: 50. These numbers differ in individual departments.
Altogether 425 foetuses with severe types of chromosomal aberrations and congenital defects were found by methods of prenatal diagnosis in 2000.

P0571. Community Genetics in the Netherlands: past and future

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In 1995, a small group of people in the Netherlands formed the “Initiative Group Community Genetics’. Community Genetics concerns the application of medical genetics to the benefit of as many people as possible and plays an active role in going out to the community. Community genetics involves a wide scope of activities, including genetic education, genetics in primary care, genetic screening, and registration of genetic anomalies and genetics for disadvantaged groups.
Very recently, as a logical consequence, The Dutch Association of Community Genetics (NACG) was founded (2001). Its aim is to stimulate responsible applications of medical genetics in society. Promoting scientific research, encouraging public education and discussion, and organising conferences and workshops pursues this goal. At this moment the NACG has about 80 members and includes a variety of professions and disciplines, involving both research and application. General practitioners, clinical geneticists, health care workers, midwives, medical psychologists, and epidemiologists are only a few examples.
The NACG just had its first yearly meeting and the intended policy for the nearby future was discussed and approved. A leaflet has been developed, a newsletter (2 times a year) and website are planned. This is done to increase familiarity with community genetics, the NACG and its ideas, and to support the development of community genetic centres at academic universities. Furthermore, the NACG promotes public education and will be programming scientific meetings every year, as they did the last five years.

P0572. An empiric survey on biobanking in human genetics in six EU countries

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Biobanks correspond to different situations: research and technological development, medical diagnosis, therapeutic activities. Their status is not clearly defined. The aim of the work was: 1) to make a typology of the different situations encountered and 2) to investigate the way ethical implications were dealt with in various contexts. Data from a survey in 6 EU countries (France, Germany, Netherlands, Portugal, Spain, UK) have been collected in the framework of a European Research Project (EUROGENBANK). A total of 147 structures with biobanking activity were explored through questionnaire and interviews. Results: most of investigated structures belong to public or private non for profit sector, that have a key role for biobanking. This activity is increasing in all countries because few samples are discarded and genetic activity is growing. The size

of collections is variable: lots of small collections, few very large ones; purposes of collections are often research or research and healthcare mostly in the context of disease studies. Specific budget is very rarely allocated to biobanking and costs not often evaluated; samples are usually provided free of charges; gift and exchanges are the common rule. Good practice guidelines are generally followed and quality controls performed but quality procedures are not always clearly explained. Associated data are usually computerised (samples traceable or identified). Biobankers generally do not favour centralisation of samples, rather that of data. A European legal and ethical harmonisation is considered likely to facilitate international collaborations. A series of recommendations from the EUROGENBANK project have been issued.

P0573. Population Screening, Diagnosis and Care of Mentally Retarded Children with Community Genetic Approach : Indian Experience.

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Approach of detection, diagnosis, management and care of the mentally retarded (MR) children in India differs from that of the industrialized countries due to its varied socio-economic, religious, cultural and family structures. The rural, slum and semi-urban population with genetic diversity make the situation more complex by consanguinity. A community-based approach was therefore used for creating awareness about genetic components, and care of the MR children by establishing a “Referral System” with networking of primary health centres.

During 3 years’ study, screening of 5.5 lakhs of population was conducted using the existing Primary Health Post infrastructure. Training of medical, paramedical and community health volunteers (N>800), and door-step care by this trained field staff, followed by detection and referral of the MR children to the tertiary Genetic Centre - CREMERE, were the important features. The nature of the disability, causes and recurrence risk were explained through genetic counseling, emphasizing consanguinity and hereditary factors to the parents. Pregnancy monitoring and prevention was stressed in young couples.

The causative factors in total 511 MR children are discussed in view of the present health scenario, genetic expertise and limited laboratory infrastructure in India. Emphasis is given on prevention, when therapy is yet a distant hope. The significant point indicated for health policy planners was the 49% preventable factors (birth asphyxia, infections and low birth weight) and 36% genetic etiology contributing to mental retardation. Integration of genetic health care at the grass root level is functionally demonstrated in this community study.

P0574. Genetic Service For Prenatal Diagnosis In Bulgaria

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Objective: To assess a centralised model for genetic service.
Methodology: All activities related to prenatal screening, diagnosis and prophylactics of inherited disorders are concentrated in a single Centre for Maternal Fetal Medicine in Sofia. The Centre performs: 1) mass neonatal screening for PKU ; 2) selective postnatal biochemical and molecular genetic screening; 3) second and first trimester Down syndrome (DS) screening; 4) prenatal enzyme and DNA diagnosis of the common monogenic disorders and DS . The Centre provides qualified genetic counselling , DNA bank and a genetic register of high-risk families. Results: For 10 years period a total of 1109 families with clinically diagnosed monogenic disorders and DS were referred to the Centre. Prenatal DNA diagnosis were performed in 388 of them - Cystic Fibrosis-94 (24 affected fetuses, 46 carriers), SMA - 31 (5affected fetuses, 18 carriers), ß-thalassemia-39 (9 affected fetuses, 20 carriers), PKU-6 (1 affected fetus, 2 carriers), DMD/BMD -24 (4 affected fetuses, 3 carriers), Haemophilia A-13 (2 affected fetuses, 4 carriers), CMT-1 (unaffected), Down syndrome-180 (6 affected fetuses). In 80% of the cases CVS and in 20% - amniocentesis were performed.
Conclusion: For small countries with limited resources the centralised model for genetic services offers a series of advantages. The same infrastructure, communications and genetic register are used for the different screening programs with the same analytic technologies for most of the cases.

P0575. Pentaplex X polimorphisms to determine parental origin of X chromosomes in Turner patients.

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It have been reported that 40-60% of patients with Turner syndrome (TS) are monosomic for the X chromosome, the remaining cases have a structurally abnormal X or Y or are mosaics with a second cell line with a normal or abnormal sex chromosome. Experimental evidence has demonstrated that 70-80% of 45,X patients retain the maternal X chromosome, while X isochromosomes can be either paternally or maternally derived.

We studied fifthy females with Turner’s syndrome, (age ranging from 5 to 32 years) karyotyped and opportunely treated in the Pediatric center twenty-seven out of 50 were mosaics while the other twenty-three were 45,X monosomy.

The 27 females with mosaic pattern were characterised by a large variety of X chromosome with structural abnormalities and different phenotypes.

Furthermore the molecular search for Y chromosomal material on the mosaicism confirmed the cytogenetic findings.

Parental origin of X chromosomes was determined in genomic DNA by PCR using a pentaplex with the five loci amplified dye-labelled in sets of two primer pairs detected in the following size ranges: NED-AME (103-109bp) FAM-DXS101 (179 -233 bp); JOE-DXS10011 (137 -257 bp); FAM-HUMARA (255 -327 bp) and TAMRA-DXS6807 (251 - 265 bp). PCR was performed using a Trio-termoblock (Biometra), and separation of the fragments was achieved by capillary electrophoresis using an Applied Biosystems Prism 310 running GENESCAN 2.1 software.

We were able to determine that the parental origin of the single X chromosome was maternal in 90% of the monosomies.

P0576. European Molecular Genetics Quality Network (EMQN)– Developing external quality assessment for molecular genetics in Europe

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Genetic testing laboratories must answer to the individuals and families who seek their services for the quality and validity of the services they provide. In order to maintain public confidence, it is essential that the highest technical standards are met in public and private sector laboratories; that different standards between European centres are levelled out and that pressure exists to maintain current standards and improve them over time. The European Molecular Genetics Quality Network (EMQN), set up in October 1998 with funding for the European Commission’s framework IV programme, aims to address these issues by raising the standards of quality in molecular genetic testing. The EMQN organises external quality assessment (EQA) schemes and promotes best practice through the organisation of disease-specific workshops. Since 1999, EMQN has provided 28 EQA schemes in ten disease-specific areas and organised 14 disease-specific best practice workshops. The results and guidelines from these schemes and workshops are available on the EMQN website (www.emqn.org). Funding of EMQN by the European Commission finished at the end of March 2002. EMQN is continuing as a not-for-profit organisation linking expert centres in Europe and supported by the subscription of the laboratories for which it provides quality assurance services. Its advisory board will include EQA providers and expert centres.

P0577. Legal and Ethical Environment of the Estonian Genome Project

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The aim of the Estonian Genome Project is to create a comprehensive database containing the health data and genetic information. This database would be invaluable tool for finding associations between disease phenotypes and particular LD map structures. The database will be established and maintained by the Estonian Genome Project Foundation that has been founded by the Republic of Estonia. The Ethics Committee of the Estonian Genome Project observes all the procedures.
The Human Gene Research Act regulates main aspects of the Estonian Genome Project. In addition to that supportive legal documents (informed consent, material destruction protocols etc.) will be enacted by the Government. The Act is the most comprehensive legal documents in the world regulating population based genome studies. The Act stipulates the rights and duties of gene donors, data protection requirements and other guarantees for the protection of gene donors as well as liability. The Act states administrative and criminal charges for violation of the rights of a gene donor. Every gene donor if she/he has decided to participate have to sign the informed consent form, then description of state of health is prepared by filling the questionnaire together with the family physician and a tissue sample is taken. People can opt out at any moment and they have right to know (if they wish) their own health data and LD map. All research projects on the basis of this database can be performed after the project has been approved by one of the Human Research Ethics Committees.

P0578. Introduction of Iranian patients`cell bank in National Research Center for Genetic Engineering and Biotechnology

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In Iran and many countries in MiddleEast the consanguinous marriage is one of main reason for high prevalance for genetic disorders. In our center we are able to detect someof genetic dieases such as Cystic fibrosis, Galactosaemia,a-1 Antitrypsin deficiency, Myotonic Dystrophy(MD), Huntington disease(HD), Kenedy, Prader-willi, Angel-Man, LHON, MERRF, MELAS, NARP, CPEO, IBM, Leigh Syndroms, Fragile X, Deafness, Acondroplasia, NDT, SMA 1.
We are going to set up molecular methods for other rare genetic disorders.In the other hand,any disorders are very severe and affected individuals die at the first days or month of birth therefore research studies such as DNA analysis of them need more DNA.So we decide to build up cell bank in our center. This bank is a good repository for human cell lines, representing the unique and large samples of rare genetic diseases and ethnic variation of the Iranian population.We concentrate on collecting cells from individuals and family affected by autosomal recessive disorders or other rare diseases.
At present we have restored some cell lines(LCL) from diseases as following:CPEO,Deafness,CAH,Achondroplasia, Huntington`s disease,Cystic Fibrosis,Myotonic Dystrophy,LOHN,MERRF,MELAS, cytogenetic abnormalities and etc.

P0579. Rapid scanning of the RET proto-oncogene by denaturing high-performance liquid chromatography (D-HPLC): implications for genetic counselling

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Germline activating mutations of the RET proto-oncogene cause three different dominantly inherited cancer syndromes, including multiple endocrine neoplasia type 2A (MEN 2A), type 2B (MEN 2B), and familial medullary thyroid carcinoma (FMTC). Mutations, involving the somatic cell lineage, are found in about 30% of sporadic medullary thyroid carcinomas (MTC). Early detection of mutations is mandatory for genetic counselling and risk assessment in family members allowing presymptomatic testing and improvement of the disease management. The majority of the activating RET mutations affect exons 10, 11, 13, 14, 15 and 16 and are currently detected by SSCP and restriction enzyme analysis. In order to improve sensitivity, time and cost of the RET mutation analysis, we have developed a Denaturing High Performance Chromatography (DHPLC)-based protocol. In this system mutations can be determined on the basis of the melting behaviour of heteroduplexes, which elute from the column by a combination of temperature and acetonitrile gradient. We performed DHPLC in 141 MTC patients with previously characterized mutations and 35 relatives. Heteroduplex peaks were detected for each mutation tested which produced a distinct and highly reproducible DHPLC elution profile. These results indicated that DHPLC methodology: a) displays a high level of sensitivity, approaching 100% for mutations in the RET proto-oncogene; b) is suitable for rapid genetic testing of members of the MEN2 affected families; c) provides a relatively simple and accurate screening technique by exhibiting advantages over conventional mutation methods, including semi-automated analysis of 96 PCR samples in less than 12 hours and low cost .

P0580. French CF-Network of molecular genetics laboratories

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Thirty five French molecular genetics laboratories carry out CFTR studies. Each year, around 8300 postnatal studies and 240 classical prenatal diagnoses (risk: ¼) are performed. Almost 50% of the studies concern carrier screening in CF relatives and their partners; 18%, monosymptomatic forms in adults; 17%, atypical forms in children; 12%, suspicion of CF in fetuses displaying echogenic anomalies. Most laboratories screen for the most frequent mutations as a first step and, if necessary, turn to more specialized laboratories to complete CFTR studies. The French CF-network, officially recognized by the Ministry of Health, is composed of three kinds of laboratories, depending on their level of molecular expertise and their implication in the management of the network and in CF research. Those who offer complete CFTR gene analysis are directly funded by the Ministry of Health. The French network works in collaboration with the European CF-Network (coordinated by J.J. Cassiman and E. Dequeker), participating every year in the external quality assessment scheme for CF. Three workshops have been organized in France: at Creteil (April 1997), Lyon (May 1998) and Creteil (December 2001). This workshop, which convened molecular geneticists, clinical geneticists and specialists in charge of CF patients, provided the opportunity to discuss about best practices for CFTR molecular studies, taking into account the European recommendations (Eur J Hum Genet 2000, vol 8, supp 2). The main conclusions of this workshop will be presented.

P0581. The Italian national project for standardisation and quality assurance of genetic testing.

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Genetic services in Italy are characterised by a large number of Laboratories, some performing at outstanding level; however, major problems are lack of homogeneous quality standards as well as of a widespread quality assurance system.
During 1999-2000 the Italian government adopted the National Guidelines on Genetic Testing (http://www.malattierare.iss.it); priority topics include quality standardisation and implementation at both intra- and interlaboratory level. Accordingly, the first national Project for standardisation and quality assurance of genetic tests has been launched on 2000-2002. The project, financially supported by the National Health System, is co-ordinated by D. Taruscio from the Istituto Superiore di Sanità. The main activities of the Project are

quality control trials on :
1) cytogenetics (prenatal, postnatal including oncological)
2) molecular genetics (cystic fibrosis, beta-thalassemia, X-fragile syndrome, APC gene).
Eighty Laboratories have been enrolled, covering all Italian Regions; there are 6 inter-regional Working Units (WU). Decisions are discussed by the Steering Committee, including the WU co-ordinators and reference experts. Laboratories participate anonymously, identified by a code number.
Preliminary results either in cytogenetics and molecular genetics trials indicate an overall rate of diagnostic errors approximating 10%. Non-standard nomenclature is used by approximately 15% of Laboratories. The main factors explaining inaccuracies are under evaluation.
Expected results and deliverables include: a) improving quality and homogeneity of Italian genetic services, through, e.g.: reduction of interregional differences; b) improving dissemination of knowledge and quality standards among health operators; c) contributing to harmonisation of protocols; d) elaborating recommendations for a permanent programme on quality assurance.

P0582. Spinal Muscular Atrophy - A Common Inherited Disorder In Bulgarian Gypsies

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A number of private genetic disorders in Gypsy population have been described and characterized in the recent years. So far, Spinal Muscular Atrophy (SMA) has not been considered specific to Gypsy population. We have studied the molecular characteristics of a total of 38 SMA families - 30 from Bulgaria and 8 from Hungary. The Gypsy SMA patients in Bulgaria represent 35% from all patients analyzed. Our data point to a non-random distribution of the disease in the Roma in Bulgaria. The disease is confined to the Xoroxane Roma, clustered in the South-Eastern part of the country, where an ancestral SMA allele accounts for 98% of SMA chromosomes. We found that this SMA allele has diverged in three closely related conserved haplotypes, carrying different types of SMN gene deletions and conversions. Their combinations determine the varying severity of SMA disease. By contrast, the same ancestral allele is rare in Hungary. It occurs in only 50% of Hungarian SMA chromosomes and is not confined to any specific Romani group.
The high prevalence of SMA in Bulgarian Gypsies, and the existence of a founder SMA allele, raise the question of prevention among the target Xoroxane groups. However, the lack of an inexpensive, reliable, high-throughput method for the detection of deletion carriers makes population-based carrier testing programs unrealistic at this stage. Prenatal diagnosis in known high-risk families still remains the most feasible approach to disease prevention.

P0583. 4 year experiment of the Centre Médical Jérôme Lejeune to increase the link between clinical medicine and clinical research into genetic intellectual disabilities.

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The CMJL is opened since 1997, and offers an annual or biannual follow-up for patients of all age presenting an intellectual disability of genetic origin. These consultations allow a medical, psychological, speech therapy and social follow-up as well as the realization of a biologic checkup, to detect and handle the complications connected to every type of pathology. Since the opening, more than 2000 patients from 0 to 60 years old came regularly in consultation.The diseases are in descending order: Down syndromes (trisomy 21, 75 %), unexplained intellectual disabilities (15 %), other chromosomal abnormalities (4p-, 5p-, 18q-, tri 9p, Willi-Prader, microdeletion; 5 %), and monogenic diseases (fragile X, Rett's syndrome, phenylketonury; 5 %). This important patient cohort of all age allows introducing research programs. The initiative of them is or internal (double blind control clinical trial of folinic acid versus placebo in toddler Down people), or collaborative (epidemiological study on folate enzyme polymorphism; epidemiological study of solid tumor in DS; relation between cataracts, oxidative metabolism and IQ in DS). Our experience exhibits that families are ready to participate to research studies even without direct individual benefice. The therapeutic trial protocols are the most asked by families, but can take place only if the criteria of judgments are very rigorous. The validation of the psychometric or neuropsychological evaluations is possible with the number of subjects of our population and the narrow links existing between the research and the clinical follow-up.

P0584. Enabling the Translation of Genetic Research Into Diagnostic Service: A Comparison of Three Cases.

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Access and availability of diagnosis of very rare inherited diseases (1 in 10 000) presents a real problem to the patient as well as to the health-care provider. Much discussion has occurred concerning the multifarious applications of genetic research and the consequent changes that must occur in basic definitions of treatment, prevention and diagnosis, not to mention etiology. There is, however, a decided lack of clinical evidence concerning the impact of information services which function as intermediaries between centers engaged in research into very rare inherited disorders and the clinicians and patients seeking DNA-based diagnostic analyses. We present here three cases of patients or families in need of diagnosis of a putative genetic disorder. Each case presents a vignette epitomizing a common problem faced in the diagnosis of very rare inherited diseases. In addition, each vignette shows how the problem was overcome through information available on the internet (www.eddnal.com) to researchers and health care professionals. This is the first clinical presentation of the efficacy of a web-based health information service enabling access to DNA-based diagnostic services.

P0585. A novel method for human blood DNA purification using automated magnetic particle processor

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KingFisher Blood DNA kit is developed for the purification of genomic DNA from human blood using paramagnetic particles and KingFisher mL™ magnetic particle processor. The kit is based on two-step process where three different kind of magnetic particles are utilized. In the first step white blood cells are captured from whole blood using leukocyte specific magnetic particles thus leaving behind e.g. red blood cells and serum proteins. In the second step white blood cells still attached to beads are lysed in lysis/binding buffer and released DNA is isolated using DNA binding magnetic particles. Finally the purified DNA is released into elution buffer and is ready for the direct use in PCR or other enzymatic reactions. Fresh human blood (stored maximally 3-4 days at +4ºC) is strongly recommended as starting material. EDTA is recommended as an anticoagulant. Maximal sample volume is 200 µl, which typically gives 4-10 µg of high quality DNA. The approximate processing time for 15 samples is 50 minutes. The KingFisher Blood DNA kit provides a timesaving and convenient way to purify genomic DNA from human blood samples. The purification process requires no phenol/chloroform extraction or alcohol precipitation, and involves very little handling. As a result, DNA with high quality and yield is obtained for further applications, such as amplifications, digestion with restriction endonucleases and Southern blotting.

P0586. Mutation Screening in Lysosomal Enzyme Genes by Real-time Monitoring of Melting Behavior in Oligosaccharides labeled with SYBR Green

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Lysosomal storage disorders comprise approximately 40 recessive enzyme deficiencies leading to markedly reduced life span and severe deterioration of multiple organ functions. Promising recent results of novel intravenous infusion therapies with recombinant

enzyme preparations markedly increase the impact of a complete elucidation of the genotype in affected families. Due to the high number of rare or unknown mutations this includes laborious electrophoretic methods of mutation screening like Single Strand Conformation Polymorphism (SSCP) or Denaturing Gradient Gel Electrophoresis (DGGE).
We evaluated the possibility of using real-time PCR to monitor the melting behavior of fluorescently labeled oligonucleotides for mutation screening. PCR replicons of 100 bp - fragments, overlapping at a length of 12 bp were labeled with the unspecific fluorescent dye SYBR Green and the differences to wild type melting temperature were assesed in a ROCHE Light Cycler. Compared to SSCP the results could be obtained with a considerably shorter time (1,5 vs 8 hours) at a comparable specificity. The increased number of PCR reactions necessary could be reduced by the efficient optimization in real-time PCR. Within two years we were thus able to identify 80-90 % of the affected alleles in 108 patients with 4 rare lysosomal genenzyme deficiencies. 33 (55%) of 60 mutations found were so far unknown. Therefore this approach may be useful for the routine genotyping of patients with rare genetic disorders.

P 11. Genomics and Bioinformatics

P0587. Comparative mapping of human chromosome 22

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Human chromosome 22 is one of the smallest autosomes involved in many human genetic disease. Comparative mapping of human chromosome 22 has not been very well described in most unrelated species. This work was to choose homologous genes from human chromosome 22 (HSA22) and map them in cattle to understand the homology between human chromosome 22 and cattle chromosomes. In this way polymorphisms for six genes (ADSL, BZRP, CYP2D, CRYBA4, DIA1 & PDGFB) that showed strong sequence homology with their human counterparts and their locations are well known in human have been developed in cattle. Polymorphisms were developed for introns or 3'UTRs of those genes. Those polymorphisms have been genotyped and genetically mapped in cattle using International Bovine Reference panel containing individuals related to three generations. Those genes have been mapped by genetic linkage analysis in the frame work provided by International cattle genome mapping facility in CSIRO, Brisbane Australia. They have been mapped on cattle chromosome 5 and 17 and developed understandings of conservation between human chromosome 22 and cattle chromosome 5 and 17.

P0588. Molecular characterisation of pericentric inversion breakpoints on chimpanzee chromosome 19 compared to human

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Structural differences of the primate chromosomes include heterochromatin variability, inversions, and the telomeric fusion of two ancestral chromosomes, which resulted in the generation of human chromosome 2. Since genetic disparities are assumed to be responsible for phenotypic differences between hominoid species, molecular characterisation of the genomic regions harbouring evolutionary breakpoints is important. These analyses will help to understand the mechanisms involved in chromosome evolution. Additionally, the molecular definition of evolutionary breakpoints will reveal whether they overlap with human genomic regions susceptible to constitutional or somatic rearrangements and whether owing to intrinsic sequence features these breakpoints have been utilised more than once during evolution. So far, only the fusion event, which gave rise to human chromosome 2 has been characterized on the molecular level. Currently, we are investigating the pericentric inversion of the chimpanzee (PTR) chromosome 19, orthologous to human chromosome 17. Human BAC clones from 17p13 and 17q21.3 were identified, which span the inversion breakpoints. FISH with these BACs resulted in split hybridization signals on PTR19. Subfragments of these human BACs were used to isolate chimpanzee BACs, which in turn cover the inversion breakpoints. Sequence analysis of the junction and the respective normal sequences revealed that the inversion took place in 5-7kb spanning regions rich of repetitive elements and that nonhomologous recombination between Alu elements has been involved. We specified the sequence environment flanking the breakpoints, identified the genes next to the breakpoints on either site, and addressed the contribution of this inversion to the structure of these genes.

P0589. Human data in SWISS-PROT and TrEMBL

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TrEMBL was created as a supplement to the SWISS-PROT Protein Knowledgebase. Whereas SWISS-PROT is a non-redundant, manually curated protein sequence database, TrEMBL is a computer-annotated database containing the translations of all coding sequences present in the EMBL-Bank nucleotide sequence database that are not present in SWISS-PROT.
SWISS-PROT contains 7,652 annotated human sequences. 31,673 exist in TrEMBL awaiting annotation and/or merging. We aim to fully annotate and describe these human protein sequences and distribute them to the scientific community as soon as possible, thus enriching the biological knowledge of the human genome. More about the 'Human Proteomics Initiative' (HPI) at http://www.ebi.ac.uk/swissprot/hpi/ .
A human SWISS-PROT/TrEMBL non-redundant proteome set was built, consisting of 24,049 entries (from a total of 39,325 human entries). Proteome analysis was performed, offering statistical, structural, functional and comparative analyses, http://www.ebi.ac.uk/proteome/ .
As all human coding sequences are not yet in the nucleotide sequence database, the SWISS-PROT and Ensembl teams jointly constructed a draft complete non-redundant proteome, comprising 23,377 SWISS-PROT/TrEMBL non-redundant entries and additional 9,136 Ensembl predictions.
Gene sets for the human chromosomes were constructed, providing a comprehensive reference of the human data in SWISS-PROT/TrEMBL. Each set is an alphabetic listing of the genes with the HUGO approved gene symbol (or the NCBI LocusLink provisional symbol) encoded on that chromosome, together with the chromosome position, the protein it encodes and useful links to other databases, such as GeneCards, OMIM, Ensembl, InterPro and CluSTr.
Currently, 14,932 gene mappings have been made, 8,427 of which with an official HUGO gene name.

P0590. Biomax PEDANT™ Human Genome Database - Automatic and Manual Functional Annotation of the Human Genome

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Public and private efforts have begun to extract valuable information from the wealth of human genome data. Biomax Informatics systematically analyzed data for all human genes (both known and unknown) in a consistent manner to determine (possible) protein function and, when necessary, refined the annotation manually . All information has been stored in a relational database, which can be accessed via a Web-based user interface.
Using the publicly available working draft assembly of the human genome, we identified the location of known and putative genes with the exclusive Fgenesh++ software from Softberry, Inc. The Pedant-Pro™ Sequence Analysis Suite from Biomax was used to perform systematic, comprehensive and consistent analysis for in-depth functional and structural characterization of the predicted proteome. This characterization includes functional class assignments according to a functional catalogue (with more than 1500 functional categories) and assignment of EC numbers, PROSITE patterns, Pfam domains and SCOP classifications.
The PEDANT™ Human Genome Database based on the 12 December 2000 working draft assembly of the human genome and contains 44,403 gene models. Using sequence similarity to manually annotated proteomes, proteins have been assigned to functional

categories. In addition, the Pedant-Pro software performed further functional and structural analyses including similarity searches, annotation of functional domains, and assignment of keywords, EC numbers and transmembrane domains. Report pages display all important automatic and manual annotations for each protein and hyperlinks to the corresponding databases. The easy-to-use graphical user interface provides search tools as well as DNA and protein viewers.

P0591. The dynamic process of gene discovery: characterization of 19 novel transcripts from human chromosome 21

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The identification of all human chromosome 21 (HC21) genes is a necessary step in understanding the molecular pathogenesis of trisomy 21 (Down syndrome). The first analysis of the sequence of 21q included 127 previously characterized genes, and predicted an additional 98 novel anonymous genes. Recently we evaluated the quality of this annotation by characterizing a set of C21orfs and PREDs, identified by mapping spliced ESTs to the genome and only by in silico analysis, respectively. This study underscored the limit of in silico-only gene prediction, as many PREDs were incorrectly predicted. To refine the HC21 annotation, we have developed a reliable algorithm to extract and stringently map sequences that contain bona fide 3' transcript ends (3'-tags) to the genome. We then created a specific 21q Acedb that incorporates new ESTs as well as features such as CpG islands, repeats and gene predictions. Using these tools we identified 27 new putative genes. To validate these, we sequenced previously cloned cDNAs, performed RT-PCR, 5' and 3'RACE procedures and comparative mapping. These approaches substantiated 19 new transcripts, thus increasing the HC21 gene count by 9.5%. These transcripts were not previously identified probably because they are small and encode small proteins. We also identified 4 transcriptional units that are spliced but contain no obvious open reading frame. The HC21 data presented here further emphasize that current gene predictions algorithms miss a substantial number of transcripts that nevertheless can be identified using a combination of experimental approaches and multiple refined algorithms.

P0592. GENATLASThird Millenium: a database dedicated to gene and disease annotation

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We designed a new conceptual model to improve data storage and retrieval in GENATLAS

Data structure in GENATLAS$_{TM}$: The new relational GENATLAS, noted GENATLAS$_{TM}$, was developed with ORACLE and contains five major sections which consist in the GENATLAS$_{TM}$ core. The section GENE stores information on *DNA type*, *arrangement* and *structure* for a specific gene or DNA sequence. The section RNA contains data on the major gene transcript type and isoforms as well as tissue expression or associated pathologies (stored in the special section VARIANT/PATHOLOGY). Protein products are categorized according to their structures: *secondary structure*, *structure motifs/domains*, *structure homology*, etc., and their activity *categories* in the PROTEIN section. In addition, the protein EXPRESSION section displays ontologies about cellular *localization* (extra- and subcellular), tissue *type*, *stage*, etc.

The GENATLAS$_{TM}$ core is linked to three distinct directories which are the CITATION directory (containing more than 40.000 occurrences linked to Medline abstracts), the PHENOTYPE directory gathering information of mendelian disorders, susceptibility genes, somatic genetic disorders (tumors, malformations, etc.) and the LINKAGE directory includes 28.000 pairs At the moment, GENATLAS$_{TM}$ contains more than 12.000 genes, and 2.600 diseases are instantiated.

Querying GENATLAS$_{TM}$ The strategies to query GENATLAS$_{TM}$ implement multicriterion approaches according to the core sections (GENE, VARIANT/PATHOLOGY, RNA, PROTEIN, EXPRESSION) or particular fields within these sections. Actually, criterion combinatory querying constitutes one of main features of GENATLAS$_{TM}$: more than one hundred criterions can be used to query for gene structure, function and diseases.

GENATLAS$_{TM}$ would be accessible at : http://www.dsi.univ-paris5.fr/genatlas

P0593. In man trinucleotide repeats are underrepresented but some motifs are overexpanded: a novel signature for eukaryotic genomes?

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Aiming at assessing whether the reported shortage in trinucleotide repeats could be ascribed to specific motifs, such as those found dynamically mutable in man, we used the Tandem Repeat Finder program and detected approximate di-, tri- and tetra-repeats in human chromosomes 21 and 22 and in five organisms (M. musculus, D. melanogaster, C. elegans, A. thaliana, S. cerevisiae). Di-repeats are always the most represented, tetra-repeats are more represented in mammals than in the other organisms, whereas tri-repeats are consistently scarce. In man tri-repeat representation is less than ¼ of tetra-; their frequency is 6.4 repeat/Mb and their coverage 473 b/Mb. Motifs such as ACG, ACT, CCG are scarce, while the others present taxa-specific variations. Motif representation seems related to base sequence rather than content, with AA contributing positively and CG negatively. In man ACG is the least represented, ATC has the highest coverage, AAT the most frequent. Tri-repeat coverage generally increases linearly with frequency, except for AAG, ACC, AGG, ATC in man, and AAG, AGG in mouse: these repeats tend to expand in the former more than in the latter and occasionally, but never in exons, exceed copy number of 40, the observed limit for tri-repeat pathological expansions. Tri-repeat scarcity seems a feature of mammals and the variable representation of their motifs may constitute a novel signature for eukaryotic genomes: since both probably result from a structural control on DNA synthesis, the aberrant elongations found in dynamic mutations may be due to the derangement of one or more control elements.

P0594. DNA-Protein Interaction DataBaseProject: Bioinformatic DataBase of Third Genertion.

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Increasing significance of theoretical modeling in the field of molecular medicine and molecular biology makes databases (DB) for three-dimensional (3D) structures of biological macromolecules particularly important. Currently available DB for 3D structures of biological macromolecules can be divided by three generations. *First generation (G1)* is DB-depositories. To the moment Protein Data Bank (PDB) is the most successful and actively developing G1 DB. *Second generation (G2)* is specially designed DB. Nucleic Acid Database (NDB) is an example of G2 bioinformatics DB (BDB). Along with information stored in PDB this type of DB allows linking to other biological DB (PFAM; GenBank, etc.). *Third generation (G3)* is highly specialized informational systems (IS). This type is presented by classified IS like Protein-DNA Interaction and PDBSum projects that provide both detailed classification and analysis of the information stored in PDB and NDB. Currently we develop G3 BDB-DNA-Protein Interaction DataBase (DPIDB) version 3.0.1. based on Pluk (object oriented) technology.

DPIDB contains complete information on structure of DNA-protein complexes (derived from PDB and NDB) in both usual and specially designed by us data format. Our data format allowed revealing

specific features of DNA-protein interaction in complexes. The up to date classification of DNA-protein complexes is used for DPIDB. The Internet site of DPIDB v 3.0.1 (http://www.dpidb.belozersky.msu.ru) is now under construction. It will include DPIDB as well as the results of our own researches, for example, the statistical analysis of DNA-protein interactions. To the moment a review regarding two DNA-binding protein groups are presented on the site.

P0595. Aggregation of post-transcriptionally coregulated mRNAs in cytoplasmic ribonucleoprotein particles

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A cytoplasmic ribonucleoprotein (mRNP) infrastructure works in the cell as an interface between the transcriptome and the proteome, being responsible for transcript transport, localization, turnover and translational control. This complex is composed by aggregates of distinct subsets of mRNAs with regulatory RNA binding proteins, which finally interact with ribosomes and the cytoskeletal apparatus to allow protein synthesis. It has been proposed that the clusters of monocistronic mRNAs composing individual mRNPs function like poycistronic mRNAs transcribed from operons in procariotic organisms, adding with their combinatorial nature a further level of regulative complexity. If these mRNPs are really regulative units, the bound mRNAs should be endowed with uniform turnover and translation rate. Here we show that a clusterization of human primary fibroblast mRNAs based on their individual half life (measured by an high density microarray approach) produces groups of mRNAs which can be demonstrated to be colocalized, and probably belong to the same mRNP type. The understanding of this new level of cell organization has profound implications for the pathogenesis of those mendelian diseases, like myotonic dystrophy and X fragile syndrome, in which a single alteration in a mRNP component results in a complex clinical phenotype.

P0596. A Web-based SNP search tool

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We constructed a database using dbSNP and the GoldenPath data which can be accessed via a Web-browser (http://ihg.gsf.de). The search tool allows to retrieve SNPs in the genomic region of RefSeq and Ensembl transcripts and to download the genomic sequence around each SNP as given by the GoldenPath. SNPs were masked by an 'N' in the genomic sequence in order to avoid primers to be positioned across SNPs.
The database contains all entries of dbSNP mapped to the GoldenPath by the NCBI. Entries are only displayed when they hit a single chromosome, a single contig and maximally twice within a certain contig. The proportion of repeat sequence within the region -100 bp to +100 bp was calculated for each SNP. The number of exons for each gene is derived from the GoldenPath annotation.
The database can be searched by i) chromosome coordinates, ii) RefSeq or Ensembl accession numbers, or iii) rsSNP IDs. A non-redundant dataset of RefSeq and Ensembl transcripts is used for the chromosome search. All SNPs up to 10,000 bp upstream and downstream of the transcripts are displayed. Transcripts without SNPs are indicated. The search can be narrowed by the proportion of repeats and the average estimated heterozygosity.
The tool allows seven features to be downloaded: i) rsSNP ID, ii) RefSeq or Ensembl accession number, iii) chromosome position on the GoldenPath, iv) average estimated heterozygosity, v) proportion of repeats, vi) observed polymorphism (dbSNP) and vii) sequence around the SNPs either as given in dbSNP or in the GoldenPath.

P0597. UMD-STA and UMD-LMNA: Locus Specific DataBases for emerinopathy and laminopathy

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About 75 neuro-muscular disease genes have been identified so far. Among them, Emery-Dreifuss syndrome is due to defects in the emerin gene (STA on chromosome X), or in the lamin A/C gene (LMNA on chromosome 1), both coding for proteins associated to the nuclear envelope. A large clinical intra- and inter-family variability is associated to both emerinopathy and laminopathy. In addition, up to five different clinical entities are associated to LMNA mutations.
To handle and exploit the numerous data collected by the "French Clinical and Research network for Emery-Dreifuss muscular dystrophies and other nucleopathies" connected to "EUROMEN" network on emerinopathy and laminopathy (a subsidiary of the "Myo-Cluster" EC initiative), we developed a Locus Specific DataBase (LSDB). It was adapted from the Universal Mutation Database (UMD) package previously used for many LSDB's such as p53, APC, FBN1, LDLR, VHL, MEN1, ATP7B... The specific features of the present software are: (i) access to the various routines via internet; (ii) inclusion of multi-parametric analytic tools allowing optimized searching of correlations. This requires a full implementation of relevant clinical, para-clinical and biological features. We have developed a specific module to display phenotype-genotype and genotype-phenotype correlations. To date the UMD-STA database contains 130 records and the UMD-LMNA database 250 records.
This work was supported by the AFM/INSERM French Network "Clinical and research network for Emery–Dreifuss muscular dystrophies and other nucleopathies" and the European "Myo-Cluster/EUROMEN" Network.

P0598. HC Forum(r): a generic bioinformatic platform dedicated to medical genetics

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HC Forum® is the first **secure** and **generic** informatic platform dedicated to medical genetics, in which information is structured according to pedigrees and the different components of medical genetics. It includes a database accessible from a Web site. User-friendly interfaces require no specialised computer skills, but only an access with a simple Web navigator. Graphic tools allow inputting and search for information in the database. A **high level of security** includes authentication with smart cards, certified high level encryption (128 bits), medical data coding to avoid any patronymic names, and digital tattooing of images.
HC Forum® is approved by the French "Informatics and Freedom Commission " and by security audits, a medical ethics laboratory (Pr C. HERVE, Paris) and by specialized lawyer.
HC Forum® offers geneticists **private working spaces** dedicated to cytogenetics, monogenic diseases and dysmorphological syndromes, including LDDB thesaurus (R. WINTER) and international nomenclatures. It allows the inputting of **genetic records** with pedigree and a standardised **textual** description of diseases, enriched with **images** from the different clinical and paraclinical investigations.
Furthermore, HC Forum® allows **collaborative working**: (i) shared records allow several doctors to work on the same record, (ii) thematic networks allow professionals to share data related to a same disease.
HC Forum® establishes a link between medical genetics departments, research laboratories and teaching centres. HC Forum® is **academic**, free of charge and available to the international scientific and medical communities.
URL: **https://HCForum.imag.fr**

P0599. Genomic Assays Project: Building an SNP-Based Linkage Disequilibrium Map and Ready-to-Use 5' Nuclease Assays for 200,000 SNPs

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The human genome project has identified the 3 billion base human genome sequence in the past decade. SNPs in the human genome, encoding the human variance, are expected to become a powerful tool in deciphering complex traits such as disease susceptibilities and drug responses.
We describe the project to create a ready-to-use solution for association studies in the process of gene discovery. We are building a genome wide linkage disequilbrium (LD) map based on data from both the Celera and the public sequencing efforts. The SNPs selected for the LD map are validated in regards to both assay performance and allelic frequencies in at least two populations. During the project, we will make available 200,000 SNP ready-to-use assays named

Assays-On-DemandTM.
All SNP assays created in this project will be of high information content, quality and performance, based on the process we have introduced. These SNP assays will be easily accessible via a simple interface, enabling researchers to assemble the list of SNP assays tailored for their individual projects.
We will be presenting details of the genomic assays project including SNP selection , assay validation and LD map creation. The ultimate goal of this project is make genetic studies faster and more efficient.

P0600. Comparative functional genomics of an entire chromosome: coding and regulatory annotation of HC21 using the mouse genome

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Chromosome 21 (HC21) is the smallest human chromosome. Several diseases are associated with genes or genomic regions of HC21 including Down syndrome, the most common cause of mental retardation. The availability of the mouse genomic sequence gives the opportunity to combine whole chromosome homologous sequence comparisons with experimental assays to reveal novel functional regions that correspond either to putative genes or regulatory regions. We used PipMaker to perform long genomic alignments of the entire human chromosome 21q sequence with the homologous sequences from mouse chromosomes 16, 17 and 10 obtained from the Celera mouse genome assembly. 3896 blocks of >100bps in length and >70% sequence identity were extracted and fragments corresponding to known HC21 exons were excluded. From the remaining 2383 unassigned conserved blocks, 10% correspond to deposited usually singly represented human ESTs, 37% contain significant ORFs and several have strong similarity to deposited coding sequences. These suggest that many of the conserved blocks are unknown exons of known or unknown genes that were neither experimentally identified nor predicted by the sequence analysis of HC21, probably due to short length and restricted spatial and temporal expression. Conserved blocks corresponding to putative exons or regulatory regions are currently being tested experimentally for their functional role. Additionally, coding and intergenic sequences are being analyzed in order to reveal their evolutionary properties. We will present a combination of comprehensive computational and experimental analysis in an effort to improve and expand the coding and regulatory annotation of HC21 and study its evolutionary history.

P0601. Transcriptome variations in Caco-2 cells during their differentiation : a model for studying intestinal iron absorption ?

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Iron homeostasis is controlled by intestinal absorption. Many factors implicated in this absorption have been recently characterized without explaining its adaptation to the iron store of the whole organism. This adapted absorption is probably due to an unknown sensibilization of intestinal crypts undifferentiated cells. These cells differentiate during their migration along the crypt-villosity axis and would become fully differentiated enterocytes with an appropriate iron absorption capacity. Caco-2 cells, a colon adenocarcinoma cell line, seem to be a good model for studying iron effects on gene expression during the enterocytic differentiation.
A transcriptomic approach has been implemented to identify genes involved in the regulation of iron absorption in the intestine : 1536 cDNA were selected by subtractive suppressive hybridization (SSH) and spotted on micro arrays. Their sequences reveal that each array represents 700 genes, half of them with an unknown function. mRNA from Caco-2 cells at different stages of differentiation were hybridised to the microarrays. These experiments show that 400 out of the 700 genes can be classified in seven distinct expression profiles that seem to correlate with functional role, most of iron metabolism genes being indeed overexpressed in differentiated cells. The effects of intracellular iron concentration on the expression of those genes are currently being studied. These observations should enable us to better understand the mechanisms underlying primary iron overload. (genetic heterogeneity, incomplete penetrance of the C282Y mutation of the HFE1 gene.)

P0602. The use of multiple algorithms and a novel quality metric system for high throughput and accurate microsatellite- and SNP-based genotyping

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With the advent of automated fluorescent genotyping using medium-to-high throughput systems such as the ABI PRISM® 3700 and 3100 Genetic Analyzers, a fast and accurate genotyping software tool is needed to perform accurate allele calling with minimal human interventions.
The ABI PRISM® GeneMapper™ software, containing a multiple algorithm module and a novel quality metric system, was developed to meet the requirements of these automated, microsatellite- and SNP-based genotyping processes. In GeneMapper™ software, an algorithm module containing multiple allele calling algorithms - size calling, binning, allele calling, has been developed to optimize the accuracy of final genotype assignments. Coupled with a novel Process Based Quality Value (PQV) system, which contains over 20 quality metrics to pinpoint the origins of genotyping failures, GeneMapper software enables users to choose to manually examine only allele calls with low quality scores. For projects that require frequent modifications of markers, a novel panel management feature has been developed to allow on-the-fly editing of bins within markers. A collection of genotyping data has been processed using GeneScan® software, Genotyper™ software and GeneMapper™ software, and analysis results were compared. Allele calling accuracy and time required for complete analysis and data editing will be presented.

P0603. Genomic Assays To Enable High Throughput Genomics

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The draft of the human genome enables scientific discoveries in many fields including gene expression and genotyping. The challenge is to make this wealth of genome information available to the laboratory. Applied Biosystems has launched two complementary product offerings that together provide a complete solution for ready to use assays for quantification of gene expression and genotyping via SNPs These assays use the 5‘ nuclease assay with Taqman® MGB probes. All assays are provided in an easy to use single tube format. Experimental setup is simple consisting of addition of two reagents (assay mix and universal master mix) to the Target DNA. The Assays-by-Design℠ service, will allow customers to submit a target sequence of interest. Applied Biosystems uses proprietary algorithms to design an assay for each target. Following synthesis and formulation, the assay will be analytically tested for integrity before shipping to the customer. The second offering, the Assays-on-Demand™ products, will provide ready-to-use assays to pre-defined targets which customers may select by, SNP or transcript ID, gene name or attribute. Genomic assays provided by both Both Assays-by-Design℠ and Assays-on-Demand™ are provided in an easy to use single tube format. Experimental setup is minimized consisting of addition of two reagents (assay mix and universal master mix) to the Target DNA. The process is highly automatable and signal detection can be carried out unattended using the ABI Prism® 7900HT with robotic accessories
Details on the design, production and testing of these assays will be presented.

P 12. Globins

P0604. Postnatal and prenatal diagnosis of β-thalassemia by DHPLC

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ß-Thalassemia, one of the most common hereditary disease worldwide, results from a reduced or absent ß-globin chain synthesis, due to over 200 identified mutations in the ß-globin locus. The high prevalence of ß-thalassemic carriers in Mediterranean countries presses for the development of genetic counseling and postnatal/ prenatal molecular diagnosis programs. The major goal of this study was to develop a feasible protocol for the postnatal and prenatal diagnosis of ß-thalassemia in the Italian population, based on a Denaturing High Performance Liquid Chromatography (DHPLC) assay. First of all, empirical optimization of DHPLC parameters was set up in a total of 40 Italian heterozygous carriers, whose 26 different ß-globin mutations had been previously identified by ARMS-PCR. A group of 30 normal individuals was used as control. Secondly, the DHPLC optimized parameters were successfully applied to the mutational analysis of ß-thalassemic patients, both compound heterozygotes (12 subjects) and homozygous patients (4 subjects). In addition,12 chorionic villi samples were subjected to DHPLC analysis upon molecular characterization of each parental ß-globin alleles. Two other mutation detection methods (i.e. ARMS-PCR and allele-specific reverse dot-blot) plus direct sequence analysis were used in parallel to the DHPLC method, showing an accuracy rate of 100%. No misdiagnosis occurred. In summary, DHPLC has been shown to be a reliable, sensitive and rapid screening method to perform postnatal and prenatal diagnosis of ß-thalassemia in the Italian population, within 2-3 days of sampling.

P0605. New Beta-thalassemia mutations in Iranian population

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Beta-thalassemia is a major health problem in Iran and it is estimated that more than two million carriers of beta thalassemia live in Iran. Although currently the battery of 22 mutations in form of Beta strip assay (Vienna Lab) are used for mutation detection and prenatal diagnosis, the mutations of more than 20 percent of DNA samples still remain unknown by these probes. We have selected 72 samples of unidentified beta thalassemia cases, representing different ethnical and geographical areas of Iran. Samples were sequenced for beta globin gene. Results revealed one new mutation of Codon 95(A-T). We found three east-Asian mutations of IVS II-654 (C-T), Codon 24/25 (-GGT), and IVS II-850 (G-T) and also one individual with an unusual mutation of IVS I-2 (T-C), which have not been reported in non-black population. We also found other rare mutations of IVS I-130 (G-C), cd 82/83 (-G), cd 16 (-C), -88 (C-A), cd 15 (G-A), 5'UTR+22(G-A), Cap+1 (A-C), IVS II-2, 3 (+11, -2), cd 67 (T-G), cd 42/43 (+T), which are reported previously in European and Mediterranean populations. In nine carrier of beta thalassemia no mutations where found in beta globin gene , this may be result of very large deletions in beta or deletion in LCR region. These findings indicate that Iranian population shows a wide variety of thalassemia allelic distribution and also helps prenatal diagnosis program in Iran.

P0606. Differential Effects of the XMNI Site in cis to the Gg Globin Genes between Newborn Hb F Malta I Heterozygotes and Anaemic Adult Thalassaemia Homozygotes.

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The differential effects of the "regulatory" XMNI site 5' to the Gg globin gene were studied among newborn Hb-F-Malta-I heterozygotes and adult thalassaemia homozygotes.
Hb F Malta I was quantified among 135 heterozygote newborn (= 26% +/- 3.0). Genotyping of the "regulatory" XMNI site in trans excluded marked effects on the neonatal globin phenotype irrespective of gender: -- = 26.0%(n=116); -+ = 24.0% (n=19). Quantification of Hb F parameters among 7 adult anaemic thalassaemia homozygotes / double heterozygotes showed tight dependence on the XMNI genotype. Patients with b+IVS-I,6C in association with the b+ IVS-II,1A mutations and -+ at the XMNI site had high values; Hb F = 54%, Gg = 0.71, F-cells = 3.0 x 1012/l, Hb F / F-cell = 17.7pg while one patient with the same mutations and a hybrid haplotype resulting in cis – at the XMNI site (XMN I genotype = --) had lower values; Hb F = 13%, Gg = 0.37, F-cells = 0.12 x 1012/l, Hb F / F-cell = 11.4pg. The main effect appears to be on the decreased level of F-cell numbers.
Hydroxyurea increased F cell number with constant HbF/Fcell resulting in elevated HbF (% and g/dl) and total Hb (g/dl) among b+ IVS-I,6C homozygotes with XMNI --
The data suggested independent control of cellular commitment and expression of g and b globin genes subject to the XMNI genotype in adults but not in neonates.

P0607. A Step toward the finding the origin of ß-Thalassemia Mutation in IRAN

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A study of molecular lesions of beta-thalassemia in Iran showed a highly heterogenous spectrum of mutations.
More than 100 beta-globin alleles from 50 unrelated thalassemia patients were analyzed for mutations by amplification refractory mutation system (ARMS) or a reverse-hybridization Strip Assay (Vienna lab).
Haplotype analysis using 5 restriction sites was performed on the following 5 mutations: cd 44 (-C), cd 22 (7bp del), IVSI-1 (G-A), cd 36-37 (-T), IVS II-1 (G-A).
Using DNA sequencing 6 different polymorphisms in 9 frameworks were detected in the beta-globin gene of these samples.
Comparison of these frameworks and haplotypes with Mediterranean types reveals several findings: (1) the same polymorphisms are associated with more frameworks in the Iranian thalassemic population than in the Mediterranean, suggesting that the Iranian thalassemic population is more ancient than the Mediterranean; (2) the haplotype associated with the IVS II-1 mutation is in a different framework than the Mediterranean IVS II-1 mutation, indicating the probability of different origins for the IVS II-I mutation.

P0608. Testing for HFE-mutations in Hungarian patients with beta thalassemia minor

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Hereditary haemochromatosis (HH) characterised by iron overload is thought to be caused by mutations (C282Y or H63D) of the HFE gene. HH was shown to be of Celtic origin and HFE mutations spread remarkably in the Caucasian population. Beta thalassemia minor (BTM), a mild form of anaemia, also shows a characteristic geographical distribution with high prevalence in the Mediterranian region. Due to ineffective erythropoesis, BTM is associated with iron overload. The goal of the current study was to examine the potential interaction between these two genetic disorders, both with significant prevalence in Hungary. 97 unrelated, consecutive patients (males/ females:47/50, average age:33.5 years) with altered laboratory tests characteristic to BTM were examined for the presence of C282Y and H63D mutations of the HFE gene by PCR-RFLP. For the laboratory detection of the BTM phenotype, we used haemoglobin F (HbF), haemoglobin A2 (HbA2) and permeability tests. Each patient showed elevated HbA2 and either elevated HbF or altered permeability.
The HFE genotyping showed 5 (5.2%) C282Y-heterozygous and 30 (30.9%) H63D heterozygous patients, two of them compound heterozygous. The C282Y allele frequency (2.6%) was lower than the reference value for Hungary (3.8%), the difference was not significant. The H63D allele frequency (15%) was similar to the reference value (14%). We are currently collecting additional clinical data to assess the iron homeostasis of BTM-patients with different HFE-gentoypes. Our data indicate a tendency towards decreased allele frequency of the C282Y variant among patients with BTM, suggesting a possible negative interaction between the two genetic disorders.

P0609. Prevention of hemoglobinopathies in immigrant populations:The Netherlands.

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Hemoglobinopathy (HbP) prevention, achieved at high level in several Mediterranean countries, remains a neglected issue in many immigration areas of Northern Europe. The causes of such a shortcoming are related to the following factors. Lack of awareness by the majority of the population at risk and by the first (G.P.'s and midwives) and second line of healthcare (obstetricians, neonatologists paediatricians). Moreover, know-how and technology for carrier diagnostics is not always available in the laboratory. Carriers, whenever diagnosed, are not sufficiently informed about genetic risk and prevention and not referred for counselling. Finally, requests for financial support to the Dutch prevention foundation, for the implementation of a national prevention strategy, are systematically rejected. In order to offer better healthcare to the population at risk, we have created Hemoglobinopathies Work Groups intended to deal with these problems from different angles. We are trying to improve specific genetic education in the public by spreading leaflets and providing websites. The problem of professional information is tackled by symposia, seminars, publications in professional editorials, frequent training sessions and patient discussion. We are organizing a nework of labs capable of producing a diagnosis for the 6 basic traits (HbS, E, C, D, b- and a-thalassemia) and to ad trait related genetic information to positive carrier diagnoses. Our aim is to implement carrier diagnostics at the pre-marital or pre-parental stage by intervention of GP or specialist; in early pregnancy, by intervention of midwives and obstetricians and at the neonatal level (newborn screening). Preliminary results are presented.

P0610. Thalassemia Control by Carrier Screening: Indian Case Study

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Cyprus and Greece have shown that by mass screening of general population birth of thalassemic children can be prevented. In India thalassemia is a serious public health problem. Since India is a large country, therefore screening of general population for carrier status is not feasible. Hence, screening has to be restricted to high risk groups. Extended family screening (EFS) means screening relatives of the affected child for carrier status. Objective: To explore if EFS for carrier detection was feasible in India, if not, then what are the barriers to its acceptance. Methods: Hundred couples with a thalassaemic child were interviewed using a predesigned questionnaires. Parents of affected child were given information on disease and its transmission and asked if they had conveyed to their relatives the possibility of their giving birth to a similarly affected child. Results: 96 couples had no reservation in sharing information about their thalassaemic child with relatives. Relatives of 62 couples accepted risk of being carriers but only 14 families got themselves tested. Another 34 families were willing to get tested but because of non - availability of screening facility in near by town, cost of test and lack of sufficient motivation did not get themselves tested. Conclusion: Majority of parents have no reservations in sharing information about the affected child. Communication needs to be improved for all families at risk to accept the risk of having a thalassaemic child. Screening should be more readily available and high risk groups should be motivated through awareness programmes.

P0611. sp1 Gene Polymorphism in Patients with Beta-Thalassemia Major

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Beta-Thalassemia, an autosomal recessive disease, is characterized by defects in beta-globin chain production.
Nearly 200 different mutations affecting various different processes in globin gene expression, have been reported as the cause of Beta-Thalassemia, which is a serious health problem in Turkey. Patients with Beta-Thalassemia major have severe clinical symptoms including hepatosplenomegaly, anemia and deformability predominantly in cranial and facial bones. Osteoporosis is emerging as a major cause of morbidity in patients with Beta-Thalassemia major. Polymorphism at the sp1-binding site of the COLIA1 gene is thought to be an important factor in the development of osteoporosis. We amplified the region including the intronic polymorphism of COLIA1 gene to investigate sp1 gene polymorphism in Beta-Thalassemia major patients with osteoporosis. Amplified gene products were then digested by Bal1 restriction enzyme to detect the base substitution (G-A) in the sp1 binding site. 12 patients out of 15 studied were found to have an SS genotype, while an Ss genotype was observed in the other 3. The distribution of the genotypes was proportionately similar to those reported by others, although no ss genotype was observed in our study, which is the unfavorable genotype, possibly due to low number of subjects studied. Although there are findings to suggest a possible link between the COLIA1 polymorphism with increased rates of osteoporotic fracture, our results, to be extended, suggest careful interpretation of the effect of the ss genotype on bone fractures.

P0612. Molecular pathology of the delta-globin gene in the Portuguese population

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The classical phenotype of heterozygous beta-thalassemia (beta-thal) can be modified by a number of environmental and genetic interacting factors eg, the cotransmission of a delta-thalassemia determinant reducing the typical increased hemoglobin (Hb) A2 to normal or borderline values.
In this study, we have defined by molecular analysis, the beta- and delta-globin genotypes in a group of 44 individuals with beta-thal-like red cell indices but normal or borderline Hb A2 levels (2.3-3.5%), who were detected in a beta-thal carrier screening program in the Portuguese population. They were tested by ARMS for common Portuguese beta-thal mutations. Delta-globin gene mutations were identified by a PCR-SSCP scanning method followed by sequencing. We detected a beta-thal mutation in all subjects: 26 were carriers of the beta+IVS-I-6T>C mutation, 7 beta0Cd39C>T, 6 beta0IVS-I-1G>A, and 5 beta+IVS-I-110G>A. Two individuals were double heterozygotes for beta0Cd39C>T in trans with delta+27G>T, and other two individuals were double heterozygotes for beta0IVS-I-1G>A or beta+IVS-I-110G>A and delta+27G>T, respectively. Futhermore, one novel base substitution within the delta-gene promoter region, -80G>A, whose functional consequence is under investigation, was also detected in cis with the delta+27G>T, in one individual with the beta0Cd39C>T.
Another result of this study was the molecular characterization of three putative delta-chain hemoglobin variants detected by isoelectrofocusing or low-pressure ion exchange chromatography: i) delta-Cd16,GGC>CGC(Gly>Arg) - Hb B2 - was identified in heterozygosity in two microcytic and hypochromic individuals presenting also the -alfa3.7kb deletion; ii) delta-Cd136,GGT>GAT(Gly>Asp) - Hb A2-Babinga - was identified in heterozygosity in a hematologically normal individual.

P0613. Screening of beta-globin gene mutations causing beta-thalassemia in Romanian population

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Beta-thalassemia is a group of inherited recessive disorder in with a defect in synthesis of beta-globin polypeptide chain of hemoglobin is present. From people in all over the world, more than 200 different thalassemia mutations in beta-globin gene have been reported. The aim of our research is to search for distribution of beta-thalassemia mutations in Romanian population. Beta-thalassemia is a frequent genetic disorder in Romania. Therefore, the molecular diagnosis is at present a primary goal for heterozygotes screening and diagnostic confirmation. As being the only center in Romania, having such task, we started mutation screening and molecular diagnosis of beta-thalassemia in Romanian patients. Using direct detection by PCR based methods like ARMS-PCR and RFLP-PCR we have found out that the most frequent gene mutations in Romania are IVS1-6, IVS1-1, IVS2-745, cd 39, IVS1-110, cd 6 and IVS2-1(in order

to decreased frequencies). Our data indicated that the mutations identified in Romanian population are of the Mediterranean type. Furthermore, these data will be used in future prenatal diagnosis of beta-thalassemia and for current screening of specific mutations in Romania.

P0614. Identification of two novel beta thalassemia mutations and a novel compound heterozygosity in Antalya population: Hb Antalya, Cod 3 (+T)/ IVS1.110, Hb Tyne/Hb S

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Beta-Thalassemia, an autosomal recessive disease, is characterized by reduced synthesis of beta globin gene. Until now nearly 200 different mutations, affecting many different processes in globin gene expression, have been reported. b-Thalassemia and Sickle cell anemia create a serious health problem in Antalya, southern part of Turkey. In this investigation, three patients who were clinically diagnosed as b-Thalassemia minor, intermedia, and Sickle cell anemia/b-Thalassemia, were firstly screened to detect the known common beta globin gene mutations in Mediterranean Region, using reverse dot blot hybridization (RDBH), and the amplification refractory system (ARMS). However, no common mutations were observed in the b globin gene of these patients by the above mentioned methods. For this reason, DNA sequence analyses were performed to detect the sequence changes in b globin gene. We found two novel mutations; Hb Antalya, a partial frameshift mutation in codon3-5 of b globin gene leading to unstable globin chains in a patient with b-Thalassemia minor; a frameshift mutation in Codon 3 (+T) in compound heterozygosity with IVSI.110 in a patient with b-Thalassemia intermedia, and also found a novel compound heterozygosity for Hb Tyne/Hb S in a patient with b-Thalassemia/ Sickle cell anemia. We belive that such cases may be considered to be important examples for understanding both the molecular mechanisms of genetic heterogeneity and genotype-phenotype interaction in b-Thalassemia.

P0615. Hb Zürich – Altstetten (α2 142 TAA → CAT): A new hemoglobin variant with elongated α - chain analogous to Hb Constant Spring detected in a Thai woman

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During routine hemoglobin screening by ion exchange HPLC we detected a fraction eluting shortly after Hb A2 . The relative concentration was 2 % of total hemoglobin. A 30 year old Thai female resident in Switzerland showed slight anemia, no microcytosis and only minimal hypochromia. The patient was clinically healthy and showed no additional hematological abnormalities.
To identify the mutant hemoglobin, we sequenced the alpha globin genes. The alpha 2 gene showed two transitions in codon 142: the first being the common T→C Hb Constant Spring mutation. Additionally we detected a A→T transition in the third base of codon 142, leading to a His instead of Gln and the additional 30 amino acids as in Hb Constant Spring. First attempts to analyze the mutant hemoglobin by mass spectrometry were not successful. This most likely reflects the instability of the mutant molecule.
The second transition most likely occurred on the background of the Hb Constant Spring mutation and thus should exhibit the same properties with regard to combinations with alpha thalassemia(s).

P0616. A Rare Mutation of Beta-Globin Gene (IVS 2-849 A→G) at Exon 2-Intron 2 Splice Site in a Turkish Patient with Beta-Thalassaemia Major

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Beta-Thalassaemia, among the most common hemoglobinopathies in Antalya, Turkey, is an autosomal recessive disease. Mostly, point mutations on beta-globin gene causes beta-thalassaemia, only in rare cases a deletion or an insertion is resposible for the disease. Reverse Dot Blot Hybridization (RDBH) method is used for screening of common mutations and sequence analysis and silver staining were performed to detect any uncommon mutation. Here, we report a rare variant -intervening sequence 2 (IVS2) 849 A→G- in a Turkish family. While, proband's mother has IVS2.849 A→G, father has IVS1.1 genotype. The first child of the family has a IVS2.849 A→G/ IVS1.1 genotype, with beta-Thalassaemia major phenotype. Prenatal diagnosis was performed for the second gestation and genotype of the fetus was found as IVS2.849 A→G/Normal and parents decided for the continiuation of the pregnancy. Clinical findings were compared with the previous reports . This first report of IVS2.849 A→ G mutation in Turkish population, shows that there are many more mutations contributing the heterogeniety of mutation spectrum of beta-globin gene in Turkish population.

P0617. Prenatal Diagnosis of Beta-Thalassemia in Iran, eleven years study

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Thalassemia is the most common genetic disorder in Iran, with over 2 million carriers of beta- thalassemia. As a first center in Iran, we established in 1990 a prenatal diagnosis for beta-thalassemia. During this period we have diagnosed total of 478 cases (225 amnion and 253 CVS samples). Two strategies direct and indirect (RFLPs) were used for diagnosis. In direct method Arms and beta globin strip assay (Vienna Lab) using 22 common beta globin gene mutation panels specific for Iranian population were performed. Using both techniques we were able to provide a reliable prenatal diagnosis for over 96% of the pregnancies. Out of these samples 21.9% normal for beta globin gene mutation, 46.8% trait, and 27.4 % were affected.
In 3.9 % of the cases we could not determine any mutation or establishing any informative RFLP system. Our data shows very close Mendelian distributions.

P 13. Inborn Errors of Metabolism and Biochemical Genetics

P0618. Phenylketonuria In Iran, The First Report From The Historical City Of Isfahan

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Phenylketonuria (PKU) is a metabolic genetic disease, in which the phenylalanine hydroxylase (PAH) gene is mutated, resulting in the elevation of blood phenylalanine (Phe). The disease is associated with a severe irreversible mental retardation. However, early detection of the disease and the elimination of Phe from diet could prevent the PKU symptoms. In a screening program to analyze the PKU mutations in Isfahan, we examined 1611 institutionalized mentally retarded patients, ranging from 7-27 years old. Of the patients examined, 36 (2%) were positive using the Guthrie bacterial inhibition assay (GBIA). Quantitative measurement of the serum Phe showed that among the patients tested, 33 had increased level of Phe (above 10 mg/dL). Preliminary studies on the PAH mutations in these patients resulted in the detection of several mutations including the delL364, R261X, K341T, G272X and S273F. The delL364 mutation is, so far, the predominant one (about 3%). Furthermore, our analysis showed that 66% of the patient are the results of consanguineous marriages, in which the parents were first cousin, indicating the role of this kind of marriages in the prevalence of the disease in Isfahan.

P0619. Congenital Disorder of Glycosilation type Ia in a patient with Jouber syndrome and arachnodactyly

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Many forms of CDG are currently known. Serum transferrin isofocusing is the method of choice for screening however, most metabolic laboratories do not routinely use this technique. The method should be specifically requested in any child with an undefined multi-system disorder. As part of the EUROGLYCAN project we started a systemic screening for CDG in hypotonic patients. In the first hundred individuals studied we found one positive case. The two year-old patient was examined with mental retardation, profound hypotonia, random eye movements, ptosis, flat face, arachnodactyly, generalised joint hyperflexibility and lipid hepatopathy. Dysmorphic facial features, mental retardation, hypotonia, ophthalmic symptoms and cerebellar vermis hypoplasia suggested Joubert syndrome. Karyotype analysis was normal. Persistent hepatomegaly and increased hepatic enzymes raised the possibility of a metabolic disease. Serum transferrin isoelectric focusing and leukocyte enzyme studies established the diagnosis of CDG Ia (Congenital Disorder of Glycosylation). Characteristic features of CDG Ia include severe muscle hypotonia, mental retardation, ataxia, ophthalmologic involvement, failure to thrive, abnormal fat distribution, cerebellar atrophy and hepatopathy. Our patient had normal growth, normal fat distribution and no inverted nipples or strabismus, however, arachnodactyly and joint hyperlaxity were pronounced. Our case who is the first patient diagnosed with CDG-Ia in Hungary contributes to the phenotypic spectrum of this recently identifiable metabolic disorder.

P0620. Necrosis-like cell death in Prolidase Deficiency (PD) fibroblasts: molecular, biochemical and morphological characterization of five new PD cases.

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Prolidase Deficiency (PD) is a rare recessive disorder caused by mutations in the prolidase gene. It is characterized mainly by skin lesions, mental retardation and recurrent infectious. We identified the molecular defect in five PD patients. Direct sequencing of PCR amplified genomic DNA showed a G to A transversion in two siblings leading to a G448R substitution. A G+1 to C transition in one allele of intron 11, causing the skipping of exon 11, was detected in a third proband and this is the first report of a mutation in a splicing donor site of the prolidase gene. A G-1 to A transversion in intron 7 was identified in two unrelated probands and shown to cause multiple alternative spliced transcripts. Long term cultured fibroblasts from these PD patients were used to investigate the biochemical, microscopical and ultrastructural changes in affected cells. Light and electron microscopy revealed that patients' cells were more round and branched than controls. We also detected increased cytosolic vacuolization, plasma membrane interruptions, mitochondria swelling and mitochondrial matrix and cristae modifications. JC-1 labelling showed decreased mitochondrial membrane potential. An intracellular accumulation of the Gly-Pro dipeptide was revealed by capillary electrophoresis analysis. The composite data provide new insights into PD physiopathology, suggesting the activation in skin fibroblasts of a necrosis-like cellular death, which could be responsible for the typical skin lesions in this disease.

P0621. Five years of molecular diagnosis of hypophosphatasia : benefits for genetic counseling and for understanding the molecular basis of the disease

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Hypophosphatasia is an inherited disorder caused by a deficiency of bone alkaline phosphatase due to mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. The disease results in low or nil bone and dental mineralization. It is highly variable in its clinical expression, due to the strong allelic heterogeneity in the TNSALP gene. Since 1997, we studied 127 patients or families of patients from European, North-American and Australian origins and identified by sequencing 101 mutations among which 91 were not previously described. Twenty-six molecular prenatal diagnoses were performed in 22 families affected by the lethal form of the disease. The great number of mutations collected by our laboratory constituted a powerful tool for studying the correlations of genotype and phenotype, for understanding the cases of dominant transmission, and for elucidating the role of alkaline phosphatase in bone mineralization. For that, we used a panel of methodologies such as site-directed mutagenesis, 3D modeling and monoclonal antibodies directed against the protein. Our results allowed us to distinguish severe and moderate alleles, to discriminate recessive and dominant mutations, to show that the dominant effect of some mutations result from an inhibitory mechanism and to identify major functional regions of the enzyme, a result of significant importance in understanding the role of this enzyme in bone mineralization. These results have been immediately used to improve genetic counseling and molecular diagnosis of the disease and in the long term, could help to discover drugs for treatment of the disease.

P0622. Peroxisomal Disorders In Slovakia

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Introduction: Up to now about twenty inherited peroxisomal diseases has been described. Except of X-linked adrenoleukodystrophy (X-ALD), all diseases are based on autosomal recessive type of inheritance. This defect is based on peroxisome biogenesis or deficiency in individual peroxisomal protein.
Methods: Gas Chromatography/Mass Spectrometry (GC/MS) analysis of very long chain fatty acids (VLCFA), phytanic acid, pipecolic acid and urinary organic acids were used for diagnostics of peroxisomal diseases with defect in ß-oxidation route, primary hyperoxaluria I and mevalonic aciduria. In some cases digitonin permeabilisation of cultured fibroblasts was realised. For X-ALD mutations analysis cDNA sequencing was used.
Results: One case of the peroxisome biogenesis disorder and three cases of single peroxisomal enzyme deficiencies with fatal course to 3 year were diagnosed. X-ALD with variable phenotypes was found in five male patients. In two patients (brothers) of age about 30 this disease was presented as adrenomyeloneuropathy associated with adrenal insufficiency and Addison crises. In the other sibling X-ALD as childhood cerebral form and until now asymptomatic form was manifested. Among the X-ALD patients there is also a 12-year old boy with 5 year continual therapy with Lorenzo's oil. He has a slow progression of disease and nearly physiological VLCFA serum values. One case of primary hyperoxaluria I with kidney stones at 4 year and mevalonic aciduria were also diagnosed.
Conclusions: During the last five year inherited disease of peroxisomal compartment has been definitively proved for 11 patients. Two novel mutations of ALD gene were identified c.1898G>T (S6331I) and c.1979G>C (R660P).

P0623. Rapid Genetic Testing for Gaucher Disease: From Restriction Fragment Length Polymorphism (RFLP) to Reverse-Hybridization

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Gaucher disease (GD), the most frequent lysosomal storage disorder, is an autosomal recessive disease characterized by deficiency of glucocerebrosidase (GBA). Mutations in the GBA gene cause the disease and enzyme deficiency results in accumulation of glucocerebroside, mainly within cells of the monocyte/ macrophage lineage, which may lead to splenomegaly, hepatomegaly, thrombocytopenia, bone marrow suppression, and bone lesions. The disease is panethnic and has been divided into three major types on the basis of the absence (type 1) or the presence and severity of neurologic manifestations (type 2 and type 3). The most common variant of the disease is type 1, which is particularly frequent in the Ashkenazi Jewish population with an estimated disease frequency of 1 in 850 and a carrier rate to approximate 1 in 15. We have developed a reverse-hybridization assay (Gaucher Disease StripAssay) for the simultaneous detection of eight point mutations (84GG, IVS2(+1), 1226G, 1297T, 1342C, 1448C, 1504T and 1604A) and two multiply mutated alleles derived from rearrangements between the structural gene and the 16-kb downstream pseudogene (RecNciI, RecTL). The test is based on a single, multiplex DNA amplification reaction and ready-to-use test strips presenting a parallel array of oligonucleotide probes for each

wild-type and mutated allele. The Gaucher Disease StripAssay was used to screen a cohort of 91 English Gaucher Disease patients previously genotyped by RFLP. Data obtained with both assays will be presented and discussed with respect to specificity, sensitivity, design and throughput.

P0624. Oculocutaneous albinism in Germany: Spectrum of mutations in the TYR and P gene

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Oculocutaneous albinism (OCA) is an autosomal recessive disorder with heterogeneous genetic background. After extended clinical investigations of 176 unrelated patients [BKK; standardised examination protocol] with albinism we extracted DNA from blood samples for molecular genetic analyses of the genes responsible for OCA1 and OCA2. OCA1 is caused by mutations in the tyrosinase gene (TYR) encoding the key enzyme in melanin biosynthesis. We investigated the 5 exons of the TYR gene by PCR and SSCP and found variations in 66 individuals (1 conformational polymorphism in 46 patients, 2 in 17 patients, and 3 in 3 patients). OCA2 resulting from mutations in the human homologue of the mouse pink eye gene is the most common type of albinism world-wide. Analysing exons 3 to 20 of the P gene (consisting of 25 exons) by PCR and SSCP aberrant signals could be detected in 22 persons (preliminary results: for 21 patients 1 difference to the normal allele was found, one patient showed abnormalities in 2 exons). In 8 patients conformational deviations were detected by SSCP in both the TYR and the P gene. In our sample of German origin about 37% of the patients show SSCP changes in the TYR gene and 12.5% in the P gene (exons 3-20) suggesting that mutations at this locus are possibly underrepresented in Caucasians.
Here, we present SSCP data of the complete TYR and P gene. Differentiation between polymorphisms and relevant mutations was carried out using sequence analysis.

P0625. An Automated Screening Test for Multiple Mutations Associated with Hereditary Iron Overload

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Inherited iron overload is a heterogenous disorder, including “classic” autosomal recessive hereditary haemochromatosis (HH), as well as juvenile and autosomal dominant forms of the disease. The most prevalent variant among Caucasians is autosomal recessive HH due to mutations in the HFE and transferrin receptor-2 (TFR2) genes. More recently, mutations in the genes for ferroportin (FPN1/SLC11A3/IREG1) and ferritin heavy chain (FTH1) were found to be associated with autosomal dominant iron overload. In most cases therapeutic phlebotomy provides an effective and inexpensive lifelong treatment. DNA testing is now routinely used to support the diagnosis in patients with abnormal iron parameters, for the presymptomatic identification of individuals at risk, and its potential for population screening programs is currently under discussion.
We have developed a reverse-hybridization assay (Haemochromatosis StripAssay) for the rapid and simultaneous detection of 18 known mutations in the HFE, TFR2, FPN1 and FTH1 genes. The test is based on multiplex DNA amplification and ready-to-use test strips containing oligonucleotide probes for each wild-type and mutated allele immobilized as parallel lines. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot).
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P0626. Mutation Screening For Tyrosinaemia Type I

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Tyrosinaemia Type I is caused by a deficiency of the enzyme fumarylacetoacetase (FAA), the last enzyme in the catabolic pathway of tyrosine. As with most other inborn errors of metabolism, carrier status cannot be reliably excluded using biochemical markers (ie metabolites or enzymes), and is a particular issue for consanguineous families. Prenatal diagnosis by biochemical methods may also be problematic for some families.
The FAA gene maps to 15q23-25, has 14 exons, and more than 34 different mutations have been reported. There are four common mutations associated with this disorder, most of the remainder are private mutations. Of the 37 patients (from 35 families) at Birmingham Children’s Hospital, 62% of disease alleles were accounted for by these four mutations. 27% were G192T, 27% IVS12+5 G to A, 6.8% IVS6-1 G to T, and 1.4% G1009A. 68% of these patients are of Asian origin: within this group G192T is exclusive, and IVS12+5 G to A is more common than in the whole group.
Mutation screening by single-stranded conformational polymorphism (SSCP) analysis was developed to identify the remaining disease-causing mutations. 8 patients had further testing (14 untyped alleles). From this group of patients, 9 additional mutations were identified of which 6 were novel, and together accounted for 13 of the 14 disease-causing alleles. Five of the mutations were mis-sense mutations (S23P, H133R, P156Q, T325M and S352R) and one splice site mutation (IVS9-2 A to G). These findings have led to an improved approach to diagnosis of tyrosinaemia type I for clinical practice.

P0627. Tetrahydrobiopterin Deficiencies in the Maltese Population

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A higher than usual frequency of hyperphenylalaninaemia due to tetrahydrobiopterin (BH_4) deficiencies, specifically Dihydropteridine Reductase (DHPR) deficiency, is present in the Maltese population. Classical Phenylketonuria due to Phenylalanine Hydroxylase deficiency has not been identified to date.
Molecular analysis of the DHPR gene in 3 families (4 probands born to unrelated parents over a span of 4 years) has identified the G23D mutation, a previously identified mutation in 2 Italian and 1 other Maltese patient. This glycine to aspartic acid change at the 23rd amino acid in the protein alters a highly conserved amino acid in the NADH binding domain of the DHPR gene.
Population studies have shown this mutation to be abnormally frequent in the Maltese population. A heterozygote carrier rate of 2% has been established in a cohort of 400 random Maltese neonatal DNA samples. This mutation is of Mediterranean origin and is a clear example of a founder effect.
A neutral polymorphism, L132L, in the DHPR gene had previously been identified in a patient carrying the G23D mutation. This polymorphism was not present in our DHPR patients, however 6 out of 7 patients manifesting clinical symptoms typical of Dopa Responsive Dystonia (DRD) also had the L132L polymorphism in either homozygosity or heterozygosity. DRD in these 7 patients, from 4 unrelated families, is believed to be due to GTP Cyclohydrolase I deficiency - the first enzyme in the biosynthesis pathway of BH_4. Molecular and biochemical analysis are currently being carried out to identify the causative mutation in these patients.

P0628. Detection of two novel large mutations in SLC7A9 by semi-quantitative fluorescent multiplex PCR

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Cystinuria is an autosomal recessive aminoaciduria in which two clinical types have been described: I and nonI. Mutations in the cystine and dibasic amino acid transporter cause cystinuria: mutations in the heavy subunit, rBAT, coded by SLC3A1 gene, cause type I cystinuria while mutations in the light subunit bo,+AT, coded by SLC7A9 gene, cause non-type I cystinuria. Using multiplex semi-quantitative fluorescent PCR we have amplified the 13 exons of SLC7A9 together with exon 5 of DSCR1 (located on chromosome 21) as a double dose control gene. The PCR products were loaded in a 48 well acrylamide gel together with an external fluorescent size standard and run in an ABI PRISM 377 DNA sequencer. The results were processed by GENESCAN™ software. With this technique we have detected two novel large mutations in 2 Spanish families: a 5kb deletion and a 5kb duplication, both affecting exon 12, originated

by the crossing over of two 195 bp sequences, which difer by 1 nucleotide, separated by 4778 bp. This method is able to detect size differences from a single base to whole exons missing, which makes it useful for scaning genes with a small to medium number of exons. This technique requires 40-60 times less DNA than Southern blot, is reproducible and can be very useful to rapidly scan a large number of samples.

P0629. Three Novel Mutations In The Cyp21 Gene In Patients With The Classical Form Of Congenital 21-hydroxylase Deficiency

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Deficiency of 21-hidroxilase is the most frequent cause of congenital adrenal hyperplasia. Usually, CYP21 affected genes bear one of the eight mutations also present in the pseudogene CYP21P. The objective of this work was to determine new mutations in alleles that did not present pseudogene-originated mutations and in alleles of a patient with phenotype-genotype conflicting clinical form. The occurrence of three new mutant alleles is described. A mutation resulting from the insertion of an adenine between nucleotide 992 and 993 in the exon 4 was found in two heterozygous siblings. It was inherited from the father whereas the maternal allele bears the R356W. The reading frameshift changes the Lysine170 to a Serine; from this point on every amino acid changes and it creates a stop codon at the position 394. Correct segregation was confirmed by digestion with Pst I. A guanine to a adenine change in the nucleotide 166 caused G56R mutation. It is inherited from the mother and the paternal allele bears the IVS2AS,A/C-G,-12 microconversion. Segregation was confirmed by digestion with Apa I. A salt-losing patient was previously genotyped as having I172N from father and V281L from the mother, a genotype compatible with the non-classical form. CYP21 sequencing reveled that the maternal allele also bears a novel mutation. It is a G to A change at the position 391 within intron 2 splice donor consensus sequence. It is at +4 nucleotides from the GT splice donor site. An in silico analysis reveled that it completely suppressed the splice site.

P0630. Somatic and germinal mosaicism for the steroid sulfatase gene in an X-linked ichthyosis carrier

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Steroid sulfatase (STS) deficiency results in X-linked ichthyosis (XLI). It is characterized by dark, adhesive and regular skin scales and has a similar frequency in different geographic areas. A large majority of XLI patients present complete deletion of the STS gene which is located on Xp22.3. Mosaicism for the STS gene has not yet been reported in XLI. In the present study, we describe an XLI patient with complete deletion of the STS gene and his mother who harbored somatic and germinal mosaicism for this molecular defect. The family (XLI patient, grandmother, mother and sister) was analyzed through STS enzyme assay and PCR, DNA markers analysis and FISH of the STS gene. STS activity was undetectable in the XLI patient, very low in the mother and normal in the grandmother and sister. PCR analysis of the XLI patient showed a deletion from regions DXS1139 to DXF22S1 including the STS gene. FISH analysis performed in maternal oral cells and leukocytes showed one copy of the STS gene in 80% of the cells and two copies in the rest. The grandmother and sister showed two copies of the STS gene. DNA markers analysis allowed to identify that the origin of the X chromosome with the deletion of the STS gene corresponded to the grandfather. We report the first case in which the XLI is caused by the presence of a somatic and germinal mosacism of the STS gene

P0631. Tissue specific depletion of mitochondrial DNA in two boys with Alpers syndrome

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Alpers progressive infantile poliodystrophy, a neurodegenerative disease with liver dysfunction may be associated with impairment of function of mitochondrial enzymes and/or depletion of mitochondrial DNA (mtDNA). MtDNA depletion is a quantitative disturbance of mtDNA, characterised by tissue-specific reductions in mtDNA copy number. We describe two infants with liver impairment and progressive neuromuscular disease characterised by hypotonia, visual disturbances, refractory epilepsy, psychomotor retardation and profound brain atrophy. Progressive course of the disease with increased level of lactate in blood and cerebrospinal fluid suggested a disturbance in mitochondrial energy generating systems. In both patients, the analysis of hepatocyte ultrastructure revealed enormous multiplication of mitochondria of various size and microvesicular steatosis, histochemical investigations demonstrated cytochrome c oxidase deficiency. Southern blot analyses revealed markedly reduced content of mtDNA in liver (11% and 10% of the mean values in controls), brain cortex (15% and 30% of the mean values in controls) in both patients and in cultured fibroblasts (25% of the mean values in controls) of one patient. MtDNA content in muscle and heart was normal. The activities and protein amount of respiratory chain complexes were mildly decreased in isolated liver mitochondria in comparison with controls but they were normal in muscle and heart mitochondria and in fibroblasts. In one patient, mild decrease of mtDNA polymerase gamma activity was found, but its ratio to citrate synthase was normal.

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P0632. A novel approach for reliable identification of cytochrome P450 alleles by multiplex assays on Pyrosequencing™

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The P450 cytochromes are important metabolisers of a large number of pharmaceutical substances. Polymorphic variation in these genes defines different alleles, often associated with decreased catalytic activity of the gene product. The aim of this study was to set up rapid and reliable assays to identify alleles of CYP2D6, CYP2C9 and CYP2C19 by Pyrosequencing™.

Pyrosequencing™, or real-time sequencing, is a fast and accurate method for SNP analysis. Pyrosequencing AB (Sweden) manufactures the PSQÔ 96 System for analysis of up to 96 SNP assays in 10 min, and the PTP system for high throughput SNP scoring. Dedicated softwares automatically delivers genotype and quality assessment for each sample. A major advantage with Pyrosequencing is its combination of accuracy, speed and ease-of-use.

Pyrosequencing assays for determination of four single nucleotide polymorphisms (SNPs) and two deletion polymorphisms were developed to identify the functional allele *2 and five of the non-functional CYP2D6 alleles (*3, *4, *6, *7 and *8). The establishing of a multiplex genotyping procedure enabled simultaneous scoring of these polymorphisms in only two Pyrosequencing reactions. 130 patient samples were analysed and the results compared to genotyping by RFLP. Pyrosequencing correctly identified 100% of the alleles identified by RFLP and, in addition, scored two alleles that had not been assessed in the RFLP method. Furthermore, multiplex assays for identification of the alleles CYP2C9*2 and *3 and CYP2C19 alleles *2, *3 and *4 were successfully set up and verified. In summary, the Pyrosequencing approach enables reliable, cost efficient, and non-labour-intensive identification of CYP alleles.

P0633. Mapping of the gene for multiple sulfatase deficiency (MSD) by functional complementation using microcell-mediated chromosome transfer

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Multiple sulfatase disease (MSD) is a rare autosomal recessive disorder characterized by the deficiency of all lysosomal sulfatases. This causes a severe phenotype resulting from the association of features of all single sulfatase deficiencies. Cloning of the MSD gene has been hampered by absence of familial cases suitable for linkage mapping. A recent study demonstrated that the biochemical basis of this disease is a defect of a post-translational modification, which appears necessary for sulfatase catalytic function. However, no information is available on the protein performing such modification, and consequently of the gene mutated in this disease. We have used microcell mediated chromosome transfer to clone by complementation the gene associated with multiple sulfatase deficiency (MSD). Briefly, a panel of human/mouse monochromosomal hybrids containing single human chromosomes tagged with a selectable marker was used as a source of normal human donor chromosomes. All 22 autosomes human chromosomes were serially transferred from the hybrids into an MSD cell line by microcell-mediated chromosome transfer. After selection, we have isolated resistant clones and measured ARSA, ARSB, and ARSC activities. The results obtained so far clearly indicate functional complementation for all three sulfatases tested in the presence of a specific human chromosome derived from the donor cells. These results will be presented at the meeting together with submapping data which are being obtained by cytogenetic techniques and microsatellite analysis on hybrids containing radiation-fragmented chromosomes. Ultimately, we hope to identify the MSD gene by testing candidate genes from the smallest complementing chromosomal region.

P0634. Metabolite analysis for diagnosis of peroxisomal disorders: a flowchart.

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Peroxisomal disorders are an extremely heterogeneous group of genetic disorders without strict correlation between clinical picture and biochemical abnormalities. The definitive diagnosis is a complex procedure which includes metabolite analysis and enzymatic, molecular and genetic studies. VLCFA in plasma has been largely indicated as the first screening test. However the information allowed by this assay is limited to the VLCFA ß-oxidation and in many cases it is unsuitable to indicate the right diagnosis. We propose to use a diagnostic flowchart based on two simultaneous investigations: VLCFA, pristanic and phytanic acids in plasma [1] and plasmalogens in erythrocytes [2]. These analyses can be carried out at the same time on the same EDTA blood sample by GC-MS and stable isotope dilution in less of 24 hours. By the combination of the results (VLCFA profile, pristanic/phytanic ratio, plasmalogen concentration) it is possible to propose a differential diagnosis among almost all peroxisomal disorders: generalised defects, ß-oxidation defects (bifunctional protein or thiolase), X-ALD, acyl-CoA oxidase deficiency, Refsum disease, classical RCDP, DHAP-AT or alkyl-DHAP synthase deficiency. This procedure can be easily applied by a laboratory with experience in metabolite analysis without any special facility (but a GC-MS) and it has been successfully used in our laboratory from five years leading to 19 new diagnosis, all confirmed by enzymatic and molecular studies.

[1] U. Caruso et al.: J Inher Metab Dis 19 (Suppl 1):83, 1996.
[2] U. Caruso: Rapid Communications in Mass Spectrometry 10: 1283-85, 1996.

P0635. Genetic contribution of thrombophilic mutations to pregnancy complications: Fact or fiction?

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Over a period of 3.5 years, 406 patients (in the majority of the cases pregnant women) have been referred to our laboratory setting. All women were tested for the Factor V Leiden G1691A mutation, prothrombin G20210A mutation, and the MTHFR C677T polymorphism. We have categorised the patients into 9 groups to facilitate the statistical analysis, and compared them with a control group of 160 blood donors (FV Leiden mutation-allele frequency 2.5%, prothrombin mutation-allele frequency 2.2%, and MTHFR polymorphism -allele frequency 35.3%). Results are summarised in the table below. The chi squared statistical analysis (genotype frequencies) revealed statistical significance for the FV Leiden mutation in groups G ($P<0.001$), H ($P<0.001$), and I ($P<0.01$); for the prothrombin mutation in groups D ($P=0.01$), and H ($P<0.001$); and for the MTHFR polymorphism in groups B ($P=0.01$), H ($P<0.05$), and I ($P<0.001$). Our data suggest that pregnancy related thromboembolic complications (pulmonary embolism, deep vein thrombosis either prepartum or postpartum) are exacerbated by thrombophilic mutations such as FV Leiden and prothrombin, because pregnancy itself is an additional thrombotic factor for these women.

Patient group	N (number of patients)
A (1 miscarriage)	76
B (>2 miscarriages)	169
C (stillbirth)	18
D (pre-eclampsia)	16
E (increased resistance of uterine veins)	12
F (unsuccessful IVF attempts)	21
G (placental abruption)	4
H (thromboembolism)	24
I (>1 complications)	66
TOTAL	406

P0636. Early White Matter Lesions in Menkes Disease

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Menkes disease is an X-linked recessive disorder affecting the metabolism of copper. The Menkes gene product (MNK) is a transmembrane copper-transporting P-type ATP-ase considered to be the main efflux protein in human tissues. Although many patients have a severe clinical course characterised by progressive neurodegeneration, connective tissue disturbances, distinctive facial appearance, hair abnormalities, and poor outcome, variable allelic forms presenting as mild Menkes disease or occipital horn syndrome can be distinguished. Neuroimaging usually shows cortical cerebral and cerebellar atrophy as a result of progressive and extensive degeneration of grey matter, secondary demyelination, subdural accumulation of fluid, or multifocal areas of ischemic infraction. We report two infants with remarkable early diffuse white matter involvement on neuroimaging suggesting at first Krabbe disease. Diagnostic evaluation yielded low levels of serum copper and ceruloplasmin, high 64CU uptake in fibroblasts and DNA analysis ultimately confirmed the diagnosis of Menkes disease. It is concluded that Menkes disease should be considered in any male infant who presents with white matter changes, even in the absence of other distinctive features of the Menkes disease spectrum. As early diagnosis and treatment can significantly improve the outcome, Menkes disease should be included in the differential diagnosis of leukoencephalopathies.

P0637. Molecular-genetic analysis of lysosomal storage diseases in Russia.

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A special programme for the diagnosis and prevention of inherited metabolic diseases was developed in Russia. During 20 years more than 600 patients with different types of lysosomal storage diseases (LSD) were diagnosed in our Department. Since 1993 year the DNA-diagnostic for some LSD has been started. The molecular and mutation analysis has been performed in a total of 37 MPS II patients, 8 MPS VI patients, 35 MPS I patients, 8 patients with α-mannosidosis, 42 patients with Gaucher disease, 8 patients with NCL type 2, 1 patient with NCL type 3, 1 patient with Tay-Sachs disease and 1 patient with Wolman disease. The results are shown on the Table. Based on the results obtained 4 prenatal diagnostic and 5 carrier detection have been performed.

Tabl.

Disease	Point mutations	Del\ins	Structural alterations	Affecting splicing	Frequent mutations	Novel mutations
Gaucher disease	56	1	-	-	N370S 36 alleles (42,9%), L444P 16 alleles (19%)	A384N
α-mannosidosis	16	-	-	-	R750W 16 alleles (100%)	-
MPSI	37	2	-	-	Q70X 26 alleles (37%)	Q63X, A75P, P533L, Y343X, W487R
MPSII	25	3	3	6	-	DelT72,delT305,A79E,Y54D,L102R, H159P, D198G ,G224E,G340D,C432Y,D478Y,R443X, int1/ex2 a→g, ex4/int4 g→a, ex6/int6 g→a, complete IDS deletion, ex5-6 deletion.
MPSVI	14	2	-	-	R152W 6 alleles (37,5%)	L98P, del 7 bp 238-243, del T245, R152W Q160R,Q160X,R315X, L360P, Y513X
NCL type 2	13	-	-	-	R208X 12 alleles (69%)	R206H
NCL type 3	-	2	-	-	-	FsA349
Tay-Sachs disease	-	2	-	-	-	-
Wolman disease	1	1	-	-	-	-

P0638. New mutations in Russian patients with X-linked adrenoleukodistrophy.

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X-linked adrenoleukodistrophy (X-ALD: McKusick 300100) is severe neurological disease associated with elevated levels of very long chain fatty acids (VLCFA) resulting from a deficiency in peroxisomal VLCFA b-oxidation. X-ALD gene has been mapped to Xq28. The gene encodes a peroxisomal membrane transporter protein of unknown function. More than 360 different mutations of the gene have been reported and most of them are unique.
Eleven patients were diagnosed by using VLCFA analysis. The mutation analysis has been performed in 8 Russian patients with childhood cerebral form X-ALD. 6 different mutations have been identified using such methods as PCR-SSCP analysis and direct nonradioactive sequencing. Three of them (R152L, Y296C, and S606L) have previously been reported. Three mutations detected were novels ones: fs164 (c494delG), L138del or L139del in exon 1 and D555N in exon 7 . In one family with two affected boys the mutation fs164 (c494delG) was found in both sibs. But the c494delG has not been detected in DNA sample extracted from leukocytes of the mother. We suggest that the mother has germline mosaicism. The same situation was previously described for other X-linked disease but not for X-ALD (J. G. Gleeson, Am. J. Hum. Genet. 67:574-581, 2000).

P0639. Expression and characterization of several glucocerebrosidase mutations causing Gaucher disease in Spanish patients.

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Gaucher disease is a lysosomal storage disorder characterized by the accumulation of glucosylceramide. This accumulation is a consequence of the reduced activity of the lysosomal enzyme glucocerebrosidase. Several different mutations in the glucocerebrosidase gene were identified in the Spanish Gaucher disease patients, some of which were new. In order to characterize the resulting enzyme, these mutant alleles were expressed in an improved baculovirus system. This novel gene expression system allows rapid and efficient generation of recombinant baculovirus DNAs by site-specific transposition in E. Coli. In particular cDNAs corresponding to the following mutated alleles were studied: P182L, N188S, R257X, Y313H, E326K, N370S, P391L, N392I, I402T, D409H, L444P, N188S+E326K and E326K+L444P.
Expression of wild type cDNA results in an overexpression of acid b-glucosidase activity (mesured with 4MU-b-glucoside as substrate) of 6 to 10-fold the value of control fibroblasts. In the case of I402T, D409H, N370S, L444P, N188S+E326K and E326K+L444P mutations, expression studies revealed an activity lower than 30% of the wild-type values. Mutations N188S and E326K expressed separately show an activity greater than 30% of the wild-type values. In contrast, P182L, R257X, Y313H, P391L and N392I mutant alleles present no significant activities. Some kinetic properties were also analysed and western blots were carried out.

P0640. Mucopolysaccharidosis Type I in Taiwan

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Mucopolysaccharidosis type I (MPS I) is a rare autosomal recessive disorder caused by deficiency of the lysosomal enzyme, a-L-iduronidase. It represents the prototype of all MPS. The spectrum of its phenotypes ranges from severe (IH) to intermediate (IH/S) to mild (IS). We have identified 9 MPS I patients, 6 males and 3 females with age from 5 to 37 years old, and 6 of them are IH/S and 3 are IH. The diagnosis is achieved by physical examination and 3 sequential laboratory tests, which are the quantitative detection of excessive excretion of glycosaminoglycans (GAGs) in the urine, urine GAG qualitative 2-D electrophoresis, and finally demonstration of the specific enzyme defect in leukocytes and/or cultured fibroblasts. Molecular investigation was also performed on 7 patients. And 11 mutations, MI1 (G to A transition in the initiation codon ATG), A79V, Y343X, L346R, T364M, Q584X, R619G, 388-3c→g, 1447del 27 and 1474ins15, were detected from 14 alleles. Mutation detection of the 2 newly diagnosed patients from one family is to be done later. Bone marrow transplantation has been suggested, but none of these patients received the treatment. The clinical findings of the 9 MPS I patients in detail will be presented in the meeting.

P0641. Generation of a knock out model for non type I cystinuria

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Cystinuria (OMIM 220100) is an autosomal recessive disease with an average prevalence of 1/7000. It is due to an impairment in the renal and intestinal absorption of cystine and dibasic amino acids. Mutations in SLC3A1 cause type I cystinuria, whereas mutations in SLC7A9 cause non-type I cystinuria.
Here we describe the identification of the murine ortholog of SLC7A9 and the generation of a knock out model for non-type I cystinuria. The complete open reading frame of the mouse Slc7a9 was obtained with overlapping IMAGE clones. After screening a genomic library from the 129 murine strain, a clone with a15.7 Kb insert was identified, containing the first 11 exons (out of 13) of Slc7a9.

Homologous recombination was performed in E14.1 ES cells (derived from 129 embryos) and two clones were isolated, which were heterozygous for a mutation in Slc7a9. The mutation substitutes exons 3 to 9 for the neomycin resistance gene. The resulting gene encodes a protein that is truncated before the first of the 12 predicted transmembrane segments. The recombinant cells where microinjected into C57BL/6 blastocysts, and several chimeric mice were obtained. They were mated to C57BL/6 mice, and the resulting heterozygotes were intercrossed to give a F2 with the expected mendelian genetic ratios. Mice homozygous for the mutation are fertile and do not present any apparent external problem at leastuntil their current age (11 weeks). The biochemical and morphological characterization of this mouse model is in progress.

P0642. Novel mutation of human OCTN2 carnitine transporter in a patient with severe ischaemic heart disease

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Novel mutation of human OCTN2 carnitine transporter in a patient with severe ischaemic heart disease

Primary carnitine deficiency is an autosomal recessive disorder of fatty acid oxidation resulting from defect of the tissue carnitine transport. Impaired carnitine transport can be caused by mutations of the carnitine transporter gene SLC22A5 encoding the organic cation transporter (OCTN2), a Na^+ dependent carnitine transporter. It has been already recognized that the OCTN2 mutations are associated with different phenotypic presentations, and differences can be observed even in patients with identical mutations. However, cardiac symptoms are always present in the affected individuals, and there are indications that certain mutations can also be associated with heart problems even in heterozygotes. In a female patient with moderate cardiomyopathy myocardial infarction developed at the age of 45, and due to the persisting coronary insufficiency, later an artery bypass grafting was necessary by open-heart surgery. At the age of 70 myocardial infarction developed again. Examination of the SLC22A5 gene revealed a C-T transition at the 15 np of the exon V (in a heterozygote form) which is associated with serine phenylalanine replacement at the amino acid position 280, which has not been reported elsewhere. This amino acid exchange means a replacement of an aminoacid with potential functional residue. Whether this mutation plays a role in or contributes to the pathology or can be at least regarded as a mutation which generates an additional susceptibility, remains to be elucidated.

P0643. Flavin-containing monooxygenase 3 deficiency – genotypes and phenotypes

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Flavin-containing mono-oxygenase 3 (FMO3) is required for N-oxidation and detoxification of many endogenous and exogenous compounds including biogenic amines and several common drugs. Individuals with severe FMO3 deficiency have a constant fish-like body odour (fish odour syndrome) due to insufficient N-oxidation of trimethylamine (trimethylaminuria), whilst mild deficiency is associated with transient or intermittent malodour. So far, seventeen mutations or amino acid variants of FMO3 have been identified. A common FMO3 gene allele [E158K,E308G] (homozygous in 4 % of the German population) was shown to be associated with reduced FMO3 function. We now report the result of more extensive biochemical and molecular studies, using a DGGE mutation scanning method, in individuals with constant or intermittent malodour. Malodour in two patients was related to carnitine treatment, a well-known but previously unexplained side effect. Nine novel mutations in the FMO3 gene were identified; all but one are missense mutations that may leave residual enzyme activity. Mild mutations were identified in combination with severe mutations, or in homozygous state, in patients with mild variants of trimethylaminuria as well as patients with fish-like malodour under carnitine treatment. In summary, mutations in the FMO3 gene cause a broad spectrum of phenotypes ranging from severe fish odour syndrome to mild enzyme deficiency that is common in the general population but only intermittently associated with malodour. In view of the important metabolic functions of FMO3, its mild deficiency may play a role as susceptibility factor in various pharmacological and other pathophysiological conditions.

P0644. Mitochondrial DNA mutations in Russian patients with different forms of mitochondrial diseases

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During last 4 years 34 patients with mitochondrial diseases were investigated in our Department. Based on clinical data the patients were divided on four different groups: 10 patients with MELAS syndrome; 11 patients with KSS; 4 patients with MERRF syndrome; 8 patients with LHON.
Molecular-genetic analysis revealed that the patients with MELAS have A3243G mutations (n= 6), del 4977 (n=2) and other deletions mtDNA (n=2). In group of patients with MERRF syndrome mutations A8344G (n=1), del 4977 (n=2) and other deletions mtDNA(n=2) were found. In group of patients with LHON mutations G3460A (n=1), G11778A (n=6), T14484C (n=1) were found. Different size deletions of mtDNA were detected in patients with KSS (n=5), 6 patients with deletions detected had "common deletion" 4977bp. In two patients from this group the point mutation A3243G was also found.
The phenotypes of 8 patients did not correspond to the genotypes detected: 3 patients with MELAS and 3 patients with MERRF had different deletions, 2 patients with KSS had point mutation A3243G.

P0645. Determination of DL-pipecolic acid in body fluids by gas chromatography-mass spectrometry

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Background: Pipecolic acid (PIPA) is a metabolite of lysine degradative route. PIPA pathway accompanies the major saccharopine pathway of L-lysine degradation, being prominent only in brain, while it is obligate for D-lysine. Hyperpipecolatemia is found as an overflow effect in familial hyperlysinemia. PIPA accumulates in body fluids in some disorders of peroxisome biogenesis leading to deficient activity of L-pipecolic acid oxidase, too.
Objective: Simple quantification of PIPA in body fluids as a tool for biochemical diagnostics of inherited metabolic diseases.
Methods: Gas chromatography- mass spectrometry determination of PIPA as a N-ethoxycarbonyl ethyl ester prepared by reaction with ethylchloroformate in the presence of ethanol and pyridine. Reaction was performed in the matrix of deproteinized (acetonitrile-ethanol) biologic sample after extraction of neutral lipids (hexane) without preliminary isolation of amino acids. A capillary column with non-chiral phase was used.
Results: N-ethoxycarbonyl ethyl ester of PIPA was easily noticeable in the vacant area of chromatogram. Detection limit for L-pipecolic acid standard solution was 0,1 $\mu mol.l^{-1}$ under used conditions. Physiological values for PIPA concentration in serum and urine of patients without peroxisomal disorder were determined. In a male child (6 months) suspected for generalised peroxisomal disorder the serum PIPA level reached 101 $\mu mol.l^{-1}$, with urine PIPA level of 55,1 mmol/mol creatinine.
Conclusion: A simple and reliable method for determination of DL-pipecolic acid in body fluids is presented. It has the advantage of simultaneous analysis with other amino acids in one run and so it may be a component of a screening program.

P0646. Mitochondrial DNA deletions in Iranian patients.

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We report herein on 9 Iranian patients with mitochondrial DNA (mtDNA) deletions, found among 11 patients with chronic progressive external ophthalmoplegia (CPEO). Of the 11 patients, who reffered to our lab, 6 had CPEO, one had Kearns-Sayre syndrome and 3 had IBM and one reffered for mitochondrial myopathy. The authors

investigated DNA extraction from blood samples for occurrence of mtDNA deletions by multiplex PCR analyses. Four patients had multiple deletions. Five patients showed single deletions, 7 or 5 kb deletions; 3 of them had the same 'common deletion' of 4977 bp. There was no correlation between clinical severity, and the size of the mutated mtDNA, suggesting that there are still unknown factors influencing the disease phenotype.An etiologic association between the somatic multiple mtDNA deletions in CPEO and clinical manifestations other than the myopathy has so far not been demonstrated. For more investigation muscle biopsy took from patient's. Because of the mtDNA deletions reported mostly in the patient's muscles than blood samples, we are going to analyses muscle biopsy from patients by more sensetive methods such as southern blot and expand long PCR.

P0647. Statistical Evaluation of the Aminoacidopathies Diagnosed in the Laboratory of Human Genetics of Cluj County Hospital, Integrated in the Department of Cell and Molecular Biology of "Iuliu Hatieganu" University of Medicine and Pharmacy from Cluj-Napoca, Romania, Between 1981-2001

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The exact incidence of many inborn errors of metabolic diseases (IEMD) are unknown in many parts of the world. The normal pathways of amino acids could be affected by deficiences of enzymes with accumulation of toxic substances or by the deficiences in transport systems, because several specific transport systems ensure virtually complete (re-)absorbtion of amino acids in gut and kidney. These defects of transport systems are often detected only through elevation of the respective amino acids in urine with normal (or low) values in plasma. In the Laboratory of Human Genetics of Cluj County Hospital were performed plasma and/or urine amino acid analyses by two-dimensional thin layer chromatography procedure (Wadman & al. 1981) in children suspected for having perturbances of amino acid metabolism. Thus, a number of over 1800 samples from patients reffered by the Neuro- Psychiatry Clinic and other Paediatric Services from Cluj-Napoca were analysed between 1980-2001. Most of the samples (74.2%) were reffered by the Department of Paediatric Neurology of Cluj County Hospital. Out of the total samples, 56 cases were diagnosed as *hyperphenylalaninemias/ phenylketonurias*, one case of *oculocerebrorenal Lowe syndrome*, 4 cases of *cystinurias*, 28 cases of *unspecified generalized hyperaminoacidurias*. We also include a case of *sarcosinemia*, two cases of *histidinuria without histidinemia*, rare and still controversial genetic disorders concerning neurologic abnormalities determination. In our statistic over 26% of total samples had presented alteration of the normal pattern of amino acids chromatograms, without significance of aminoacidopathies; besides, 8.33% of total urinary samples shows excretion of *beta-aminoisobutyric acid*.

P0648. Carnitine Palmitoyltransferase 2 Deficiency: Attempt at Correlation between Genotype and Clinical presentation

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Carnitine Palmitoyltransferase 2 (CPT2) deficiency, a common autosomal recessive disease of the mitochondrial long-chain fatty acid (LCFA) oxidation, may result in distinct clinical phenotypes, namely a mild adult muscular form, and a severe hepatocardiomuscular infantile/neonatal form. This phenotypic heterogeneity remains poorly understood. We therefore undertook the analysis of twenty CPT2-deficient patients ("adult form": n=13, "infantile form": n=7) on a molecular, enzymatic and functional point of view. Analysis of the CPT2 gene by DGGE and/or direct sequencing enabled us to detect thirteen mutations including five novel ones: 371G>A (R124Q), 437A>C (N146T), 481C>T (R161W), 983A>G (D328G), and 1823G>C (D608H). Taking into account our results and the literature data (33 mutations) it appears that: 1/the combinations of allelic mutations identified in the muscular form have never been detected in the hepatocardiomuscular one, 2/ in adult CPT2-deficient patients, at least one of the two mutations constantly lies in exons 1-3, while 3/ all "severe" missense mutations observed in the infantile form of the disease are constantly located in exons 4-5 of the CPT2 gene.
Moreover, it appears that the difference in severity between the adult and the infantile phenotypes is clearly related to a difference in their respective levels of mitochondrial LCFA oxidation, and in a less extent, to their residual CPT2 activity in lymphocytes and/or fibroblasts. Understanding the relationship between the type of CPT2 mutation, the residual CPT2 activity, and the resulting LCFA oxidation level remains the key point for unravelling the genotype-phenotype correlation.

P0649. Identification Of 14 Novel Mutations In The Slc3a1 Gene In Spanish Cystinuria Patients And Functional Analysis Of The Mutation L89p

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Cystinuria is a heritable disorder of amino acid transport characterized by the defective transport of cystine and the dibasic amino acids through the brush border epithelial cells of the renal tubule and intestine tract. Initially, three types of cystinuria (I, II, and III) were described based on the urinary excretion of cystine and dibasic amino acids in obligate heterozygotes. The SLC3A1 gene -coding for a high subunit of the heteromeric amino acid transporters (HATs), rBAT- is responsible for type I cystinuria, whereas the SLC7A9 gene -coding for a light subunit of the HATs, b0,+AT- is involved in determining non type I (before types II and III) cystinuria. We have screened the entire coding sequence and the intron/exon boundaries of the SLC3A1 gene in 50 spanish cystinuria patients by means of single strand conformation polymorphism (SSCP) and DNA sequencing.
We identified 14 new mutations in SLC3A1 that increases the number of mutated alleles so far characterized in this gene to 88, and accounts for 83% of the type I chromosomes studied.
Functional analysis of the mutation L89P -located at the unique putative transmembrane domain of rBAT and affecting a conserved amino acid residue- in Xenopus oocytes and after coexpression with b0,+AT in HeLa cells, revealed significant residual transport activity and impaired maturation and transport to the plasma membrane, suggesting a trafficking defect.

P0650. The clinical and genetic characteristics of congenital plasma dopamine beta-hydroxylase deficiency: a severe orthostatic syndrome

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Introduction: Dopamine ß-hydroxylase (DßH) catalyses the conversion of dopamine into norepinephrine. Plasma DßH deficiency has been associated with a severe congenital orthostatic syndrome with absent plasma (nor)epinephrine. Low plasma DßH, however, also occurs in 5% of normal subjects having normal (nor)epinephrine. The genetic basis of symptomatic DßH deficiency has never been investigated.
Methods: We sequenced the DßH gene in two unrelated patients with DßH deficiency and an orthostatic syndrome. We determined plasma DßH in 49 healthy blood donors to identify asymptomatic individuals with low plasma DßH activity to sequence their DßH gene.
Results: Two mutations uniquely associated with plasma DßH deficiency and absent plasma (nor)epinephrine were found in the

patients with the orthostatic syndrome. One patient was homozygous for a splice site mutation (IVS1+2 T>C), and the other was compound heterozygote for this splice site variant and a deletion of base 575.
In blood donors with low plasma DßH activity we found a mutation at -1021, immediately upstream of the transcription initiation site. Individuals homozygous for
-1021T had almost absent plasma DßH activity.
Conclusions: Our study is the first to describe pathogenic mutations in the DßH gene. It defines the genetic basis of DßH deficiency with absent plasma (nor)epinephrine leading to an orthostatic syndrome. It also explains the genetic background of asymptomatic DßH deficiency. The concurrence of homozygosity for the DßH -1021 T-allele with low plasma DßH in healthy individuals suggests that the -1021-locus determines secretion of DßH into the blood without interfering with normal catecholamine synthesis.

P0651. Genetic Screening for Simultaneous Diagnosis of 101 Inborn Errors of Metabolism (IEM) in Critical Neonates by Gas Chromography / Mass Spectrometry (GC/MS).

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Newborn screening, recommended by WHO, is a preventive public health strategy to decrease morbidity and mortality in infants. India lags behind in introducing such health programs due to different national health priorities, and genetic screening is also perceived as expensive and concerns rare disorders.
GC/MS was first applied in 1966 worldwide in the diagnosis of IEM because of its accuracy, sensitivity and power of analyzing multiple compounds simultaneously, but was introduced in India through the present study in 1998. Matsumoto's method of GC/MS analysis for 101 metabolic disorders of amino acids, organic acids, sugars, sugar alcohols, sugar acids, nucleic acids and nucleic acid bases was used. The air dried urine filter paper offered an easy mode of transport from small towns and rural areas having limited health and laboratory infrastructure.
We report 16.5% (93 of 565 referrals) metabolic abnormalities in children, while 29.7% (19 of 64) in high-risk neonates. The genetic factors in neonates were consanguinity (26%), death of earlier sibs (21%) and h/o mental retardation (5-8%). Convulsions (37%), respiratory distress (26%), lethargy (21%) and refusal to feed (21%) were the predominant clinical manifestations.
Genetic counseling to the family was done explaining the role of hereditary, consanguinity, recurrence risk, scope of prenatal diagnosis to prevent IEM in their families. India, being diverse in its socio-cultural, religious and racial background, the importance of GC/MS is indicated in establishing its genetic epidemiology, which is currently lacking. The few cases highlighting the application of biomedical mass spectrometry in genetic screening will be illustrated.

P0652. Determination of HLCS genomic structure and mutation identification in four patients with HLCS deficiency

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Holocarboxylase synthetase (HLCS) deficiency is a rare AR biotin metabolism disorder caused by absent or reduced HLCS enzymatic activity. This enzyme catalyses the biotinylation of the four human biotin dependent carboxylases. Its deficiency leads to biotin-responsive multiple carboxylase deficiency (MCD).
The human HLCS gene has been mapped to chromosome 21q22.1. Up to now, sequencing analyses in the HLCS gene have been performed on its 2466 bp cDNA, making the confirmation of the genetic lesions at the genomic level difficult. We report on the map of HLCS genomic structure, deduced from the DNA sequence of human chromosome 21 published by the gene card web sites http://hgp.gsc.riken.go.jp; http://bioinfo.weizmann.ac.il/cards-bin/carddisp?HLCS. All Exon/Intron boundaries were tested using genomic primer. The putative deduced 9 exons, encompassing the coding region, were confirmed and PCR conditions for amplification of all genomic fragments were set up. These data confirm that the HLCS gene contains 9 exons plus two additional exons upstream of the ATG codon.
The newly available HLCS genomic characterization allowed us to identify of 4 known and 2 new genetic lesions in four patients with HLCS deficiency. The known G581S mutation was reconfirmed with new intronic primers since an undetected intron was contained in the fragment amplified by published exonic primers which were wrongly used for enzymatic analysis at genomic level.

P0653. Mutation detection of Galactose-1 phosphate uridyltransferase(GALT) gene in Iranian Galactosemia patients.

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Galactosemia is a clinically sever and heterogenous autosomal recessiv disorder caused by deficiency of galactose-1-phosphate uridyltransferase (GALT)activity.The numerous point mutations have been identified in the GALT gene and the prevalence of the mutations in some ethnic groups have been reported indicating the differences in the frequency of the mutations amongst patients belonging to the different ethnic groups. Eleven unrelated Galactosemia families (27 DNA
samples) were tested for the presence or absence of the two common mutations of GALT gene i.e. Q188R and K285N mutant alleles, using PCR-RFLP protocol. The frequency of the mutated alleles were found to be 36% and 9 % respectively. More study is being under taken on one patient with confirmed Galactosemia who is heterozygote for K285N mutation and most probably demonstrating a case of compound heterozygote.
More investigation will be carried out on Iranian patients with biochemically confirmed galactosemia, concerning detemination of eight common alleles
including Q188R, K285N, S135L, L195P, Y209S, F171S, Q169K and X380R alleles in the collected samples and the frequency of the mutations in Iranian galactosemia patients will be reported.

P0654. Molecular pathology in Czech patients with porphyria variegata

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Porphyria variegata (PV) is an autosomal dominant acute hepatic porphyria caused by a partial defect in heme synthetic enzyme protoporphyrinogen oxidase (PPO). PV is clinically characterized by acute attacks of neurovisceral crises and/or dermatologic manifestation. The clinically manifest patients show typical plasma fluorescence at 626 nm, have increased fecal porphyrins and, during acute attacks, have increased porphyrin precursors in urine. The activity of PPO in heterozygous patients is about 50%. The human PPO cDNA and gene has been cloned and mapped to chromosome 1q22-23. The gene spans about 5 kb, contains 13 exons, the cDNA encodes a protein of 477 amino acids.
We have studied 6 unrelated Czech families with clinically and biochemically diagnosed PV. Four new mutations were detected: one deletion (1393 del 8 bp), two missense mutations (W227G, C459Y) and one splicing mutation (IVS 6+1 G®A). Although the relationship is not known, the deletion 1393 del 8 bp was identified in two of these families. In the last family the already described deletion 1177 del G was found. The pathological significance of missense mutations was determined by their expression in prokaryotic system. Both these missense mutations resulted in the absence of PPO activity in E. coli which is in good agreement with an expected 50% activity in the heterozygous state. Availability of screening for all PV families will ensure correct diagnoses in all gene carriers and prevents life-threatening porphyric attacks. (Supported by Charles University grant GAUK 5/200/c and LN00A079 from MŠMT of Czech Republic)

P0655. Genetic Heterogeneity In Italian Anderson-Fabry Families

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Fabry disease is an X-linked recessive disorder of glycolipid metabolism resulting from a deficiency of the lysosomial enzyme α -galactosidaseA(GLA). The reduced enzyme activity leads to the progressive accumulation of glycosphingolipids throughout the body, particularly in: skin, kidney, nervous system, eye, heart. The α-galactosidaseA gene is located at Xq22 and consists of seven exons; more than 150 mutations have been identified in unrelated Fabry subjects. Our work was conducted to detect mutations in ten unrelated Italian families. All the subjects have been estimated clinical to renal, cutaneous, cardiac and neurological level; in all it has been executed the dosage on blood of α -GLA-A. Up until today, we identified seven affected males and eleven female carriers. In two families the study is still in progress, in one family we identified no mutations. All of the exons are small enough to allow amplification by the polymerase chain reaction (PCR) and investigation of sequence changes by Conformation Sensitive Gel Electrophoresis (CSGE) analysis and sequencing. We have detected 9 mutations: 7 single base substitutions (6 missense and 1 nonsense), 1 small deletion (delCT ex7) and one splice site alteration (IVS4-16 G →A).The missense mutations identified are G35R,R112C,R227Q,R301P and D313Y.In a family it has been characterized in all the males affected and the females carriers the mutation missense in exon 6 (R301P) and one mutation of splicing in intron 4 (IVS4).This study confirm the elevated heterogeneity of the mutations in α-GLA-A gene in patients with Anderson-Fabry disease that accompanies to one extreme clinical variability.

P0656. Molecular analysis of three Czech patients with 3-hydroxy-3-methylglutaryl CoA lyase deficiency

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Mitochondrial 3-hydroxy-3-methylglutaryl CoA lyase (HL, EC 4.1.3.4) catalyzes the last step of leucine catabolism and the ketogenic pathway. Deficiency of HL caused by mutations in HL gene leads to 3-hydroxy-3-methylglutaric aciduria (HA) an autosomal recessive metabolic disorder. Three unrelated patients with HA were diagnosed in our Institute. The diagnosis was confirmed by measurement of enzyme activity HL in lymphocytes and/or fibroblasts at all three patients and their family members.
To confirm the diagnosis at molecular level we have directly sequenced PCR or RT/PCR products amplified from probands gDNA or cDNA. In two cases we have found known homozygous missense point mutations 698A→G (H233R) and 122G→A (R41Q), respectively. In the last case, 3-years old girl, we have found heterozygous mutations H233R/del41G. Del41G (Pro9fs(-1)) is a novel one base deletion in exon1, which leads to a frameshift and premature stop codon after 32 aminoacids. Subsequently we have set up PCR-RFLP assays for mutations H233R and del41G to verify results and to determine mutations in family members. The results of molecular analysis correlate with measured enzyme activity.

P0657. DNA analysis of the phenylalanine hydroxylase gene in Puerto Rican phenylketonuria patients

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Phenylketonuria (PKU) is an autosomal recessive metabolic disorder caused by mutations in the phenylalanine hydroxylase (PAH) gene. The PAH gene harbours many mutations (~ 400 variant alleles), which cause various degrees of phenylalanine hydroxylase deficiency. Missense mutations, represent ~ 60% of the mutations so far recorded. The Puerto Rico Newborn Screening Program has found that the incidence of PKU in the island of Puerto Rico (PR) is 1:18,000 on average. The most frequent hyperphenylalanemia in PR is classic PKU. In the present study we screened 15 classical PKU Puerto Rican patients for mutations by PCR amplification of all exons followed by DGGE and/or SSCP analysis and direct DNA sequencing. We found several polymorphic sites in exons 4, 7, 11, and 12 and a novel frameshift mutation in exon 4. The novel mutation was found in one patient with a homozygous insertion of an A nucleotide at codon 120. Two patients were homozygous for the E280K mutation and another patient was homozygous for the R176X mutation. The E280K mutation has been found in high frequency among Cuban PKU patients, whereas the R176X mutation was found previously in Southern Europe PKU patients. Some patients carried missense mutations in addition to another homozygous mutation in the PAH gene. NIH-NIGMS grant R25-GM61838 and RCMI grants G12RR03051 and 1P20RR11126 from NIH-NCRR supported this study.

P 14. Linkage Mapping and Polymorphism

P0658. The Development Of A Haplotyping System For Galactosaemia

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Classical, or transferase deficient, galactosaemia is an inherited metabolic disorder, caused by mutation of the Galactose-1-Phosphate Uridyl Transferase (GALT) gene. Previous studies have shown that classical galactosaemia has an overall incidence of 1 in 21,000 live births in Ireland, higher than elsewhere in Europe. Although there is a spectrum of causative mutations in the GALT gene, one mutation (Q188R) predominates in Caucasians. The frequency of this Q188R mutation exhibits an East to West gradient of increasing frequency across Europe, peaking in Ireland (89.1%). This raises questions as to the origins and introduction of the Q188R mutation to Ireland.
Through a combination of bioinformatics, PCR, dHPLC and Restriction Fragment Length analysis (RFLP), four novel Single Nucleotide Polymorphism (SNPs) and three flanking Short Tandem Repeat (STR) markers linked to the GALT gene have been identified. A haplotype system for Galactosaemia has been established through the combination of these markers with two previously known SNPs. Sixty Irish control DNA samples have been analysed demonstrating that 8 distinct haplotypes exist in the Irish population. The analysis of the haplotype backgrounds of the common GALT gene mutations present in the European population is now underway. This should help resolve the origins and migrations of the GALT gene mutations.

P0659. CA repeats in the first intron of the CFTR gene in cystic fibrosis patients and healthy Latvians

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A dinucleotide CA repeat within intron 1 of the CFTR gene, highly informative in tracing unknown mutations causing cystic fibrosis (CF) in families as well as in population genetic studies, has been identified by D.S. Moulin in 1997. The aims of our study were to analyse CA repeat polymorphism in CF patients and healthy Latvians, and to compare our results with the data on other populations. 22 CF patients, 24 their family members and 42 healthy Latvians were subjected to DNA analysis. CA repeats were studied by denaturing gel electrophoresis of amplified PCR products and three control samples were chosen for sequencing analysis. Absolute linkage disequilibrium was found between the CF mutation dF508 and 21 - CA repeat allele. When considering allele distribution, Latvian population presented a unimodal distribution, showing peak at 22 repeats. Expected heterozygosity, estimated as $1 - \Sigma p_i^2$, where p_i is the frequency of the ith allele in the locus, for Latvian population was 0.696 that is lower than in other described European populations. The data obtained in our study support conclusions of E.Mateu et al. (1999) about high informativeness of this CFTR gene marker for family and population studies.

P0660. Linkage and candidate gene analysis of Vesicoureteral Reflux.

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Vesicoureteral reflux (VUR), the retrograde flow of urine from the bladder into the ureter and kidneys, 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. There is a strong genetic component to VUR but the mode of inheritance is still unknown.
We have collected 480 DNAs from 97 families with more than one child affected with primary VUR. We are using this resource to search for VUR susceptibility genes.
Candidate genes or regions are selected based on literature reviews. The uroplakins are 4 integral membrane proteins that physically strengthen the bladder wall. Based on uroplakin knockout mice studies, Hu et al., 2000 suggested the existence of a VUR subtype distinct from that caused by the deletion of the AT II receptor,. This report led to the selection of the first group of genes investigated. Two candidate regions were also investigated: chromosome 10q, based on a report of a patient with a deletion in this region associated with VUR and chromosome 1q, where markers over a 20cM region are reported to show strong evidence of linkage.
Results so far have shown evidence for lack of linkage to VUR in these genes or regions.

P0661. The mutation rates and polymorphisms of ten autosomal tetranucleotide loci in Western Siberian population

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Microsatellite loci are powerful and convenient markers for genetic studies, but it is necessary to evaluate the applicability of specific STR markers for practical aims and for description of population history. Tetranucleotide STRs have several technical advantages because of lesser prone to artificial slippage. However the mutation rates of this class of microsatellites differ significantly between loci. The purpose of this study was to evaluate the mutation rates and allelic frequencies of ten autosomal tetranucleotide loci in Western Siberian population. Allelic polymorphisms were studied in 200 – 290 unrelated individuals from Tomsk region, and mutation rates were estimated as frequency of parent-child mismatches in the segregation analysis of STR alleles in 134 families with proven paternity. We used polyacrilamide gel electrophoresis following PCR amplification for tetranucleotide loci D2S1242, D11S1983, D16S2624, D17S1185, D19S601, D20S161, D20S168, D21S1413, D21S11and D21S1435. All loci studied have an average heterozigosity > 0.65, PIC > 0,550 and amplicon sizes < 300 b.p. It is revealed five new allelic variants in four different loci in Tomsk population. We analyzed 1198 events of allelic transmission from the parents to children and detected nine de novo mutations events, that corresponds to average mutation rate of the complex of the loci studied 7.5x10-3 per locus per meiosis. No mutations were found in four loci (D11S1983, D16S2624, D20S161, D20S168), and two mutations were observed in each of three markers D17S1185, D19S601, D21S1435. Most of events were single step mutations, and mutations of paternal origin happened three times often than maternal ones.

P0662. Rapid SNP allele frequency determination to accelerate LD mapping strategies.

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Positional cloning of complex disease-susceptibility genes by linkage disequilibrium(LD)-mapping involves genotyping a vast amount of single nucleotide polymorphisms(SNPs). A general aim is to to achieve a density of at least 1SNP/10kb in previously identified chromosomal regions and to screen all coding and regulatory regions of candidate genes. A combined SNP map for a manageable region(±5Mb), may require genotyping 300-500 SNPs. Especially with such a high throughput, individual genotyping of SNPs remains expensive. Although current methods may be accurate, robust and adequate for large scale application, a cheaper and less time-consuming alternative to individual genotyping is using allele frequencies determined in DNA pools. We have developped an accurate and reproducible protocol for direct allele frequency determination using pyrosequencingTM technology in large genomic DNA pools(374 individuals). A correlation of 0.980 was measured between the allele frequency detected by this method and by individual genotyping. In the context of disease-associated SNPs studies, we compared the allele frequencies between disease and control groups. The measured difference varied with 1.5±0.9% between the two detection methods. It may be concluded that the protocol with large DNA pools could reliably detect a 4% difference between populations. Furthermore, it is economic with regard to amounts of DNA and reagents required. The method is currently used to screen a region of chromosome 2(6Mb) and 10(3.5 Mb) for SNPs with an allele frequency differences between type-2-diabetes or obesity and control groups. This allows a rapid identification of interesting SNPs for further association studies and susceptibility-gene discovery in these complex diseases.

P0663. Plasminogen activator inhibitor-1 4G5G polymorphism in stroke patients

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In the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene a common 4G5G polymorphism was described. A 4G allele is associated with increased transcription of PAI-1 protein due to different binding of transcription regulating proteins than in 5G site. This may change the fibrinolitic capacity, decreasing the ability to lyse clots. Many studies showed that 4G5G polymorphism was associated with increased risk for cardiovascular disease. Still, conflicting data are reported for cerebrovascular disease, even suggesting protective role of 4G allele.
In order to investigate relation between 4G5G polymorphism and stroke incidence, 52 stroke patients and 126 healthy subjects were genotyped by PCR-SSCP analysis. Genotype distribution among controls was: 4G4G 24% (30), 4G5G 48% (60) and 5G5G 29% (36). The allelic frequencies in control group were: 4G 48% and 5G 52%. In patients group the genotype distribution was: 4G4G 23% (12), 4G5G 40% (12) and 5G5G 37% (19). The frequencies for 4G and 5G alleles were 43% and 57%, respectively. There was no significant difference for genotype and allele frequencies between control and patient group (χ^2 test). A trend towards a lower prevalence of the 4G allele in the patients group was observed (43% vs. 48% in control group, OR 0.8, 95% CI 0.53-1.32). These preliminary findings suggest that 4G allele is not a risk factor for stroke and may even be related to its reduced incidence. Further studies are needed on a larger number of subjects to evaluate the role of 4G allele in cerebrovascular disease.

P0664. Autosomal dominant Juvenile Amyotrophic Lateral Sclerosis (ALS) and distal Hereditary Motor Neuropathy (distal HMN) with pyramidal tract signs

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Autosomal dominant juvenile amyotrophic lateral sclerosis (ALS) is a rare disorder and so far only one family has been reported and mapped to chromosome 9q34 (ALS4). The diagnosis of ALS in this

family is based on almost exclusive lower motor neuron pathology in combination with less prominent pyramidal tract signs. Atypical features include normal life expectancy, the absence of bulbar involvement and the symmetrical distal distribution of atrophy and weakness. We performed a molecular genetic study in 3 families that we had diagnosed as distal hereditary motor neuronopathy (distal HMN), i.e. distal spinal muscular atrophy or spinal Charcot-Marie-Tooth syndrome, and found linkage to the ALS4 locus.
The clinical phenotype in these 3 families of different geographic origin (Australian, Austrian and Belgian) is strikingly similar to the original ALS4 family except for a younger onset age in the distal HMN families. These data suggest that ALS4 and distal HMN with pyramidal tract signs may be one and the same disorder. In all 3 families, a disease-associated haplotype was present in all affected individuals. Genotyping data demonstrated that the families do not share a common disease haplotype. Cumulative significant LOD-scores (z > 3) are reached with seven STR markers. These results indicate that the distal HMN locus with pyramidal tract signs is located within the 5 cM ALS4 region, i.e. between the flanking markers D9S64 and D9S164. This region represents a 3.5 Mb region according to the Golden Path at UCSC.

P0665. Atb0/slc1a5 Gene. Fine Localisation And Exclusion Of Association With The Intestinal Phenotype Of Cystic Fibrosis

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Genetic heterogeneity in the 19q13.2-13.4 region called Cystic Fibrosis Modulator Locus 1 (CFM1) seemed to be associated to the intestinal phenotypic variation of cystic fibrosis (CF). The Na+-dependent amino acid transporter named ATB0 was previously found to be located in 19q13.3. In the present study, we performed fine chromosomal mapping of ATB0 on radiation hybrid (RH) panels G3 and TNG. Based on the most accurate location results from TNG-RH panel, mapping analysis evidenced that ATB0 is localised between STS SHGC-13875 (D19S995) and STS SHGC-6138 in 19q13.3, that corresponds with the immediately telomeric/distal segment of the strongest linkage region within the human CFM1 (hCFM1) syntenic region. The position in relation to the hCFM1 syntenic region, besides the functional characteristics of the encoded protein and its apparent relevance to meconium ileus (MI) led us to evaluate the possible implication of ATB0 in the intestinal phenotype of CF.
Regarding to the genomic structure, Blast-N program allowed us to determine that ATB0 gene is organised into eight exons. An exhaustive mutational study of the gene was performed by SSCP analysis in CF patients with and without MI. Several sequence variations in the ATB0 gene were identified, although none of them seemed to be related to the intestinal phenotype of CF. Even though no particular allele or haplotype in ATB0 appears to be associated to CF-MI disease, new SNPs identified should be useful in segregation and linkage disequilibrium analyses in families affected by other disorders caused by the impairment of neutral amino acid transport.

P0666. Homozygosity Mapping of a Weill-Marchesani Syndrome Locus to Chromosome 19p13.3-p13.2.

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Weill-Marchesani syndrome (WMS) is a rare disease characterized by short stature, brachydactyly, joint stiffness, and characteristic eye abnormalities including microspherophakia, ectopia lentis, severe myopia and glaucoma. Despite clinical homogeneity, both autosomal dominant and autosomal recessive inheritance with occasional brachymorphism in heterozygotes have been reported. Here we report on homozygosity mapping of the WMS gene in two large Lebanese and Saudian families. All affected individuals (n=5) fulfilled the criteria for WMS. A genome-wide search was performed using microsatellites markers at an average distance of 10 cM and revealed linkage of the disease-causing gene to chromosome 19p13.3-p13.2 (Zmax = 5.99 at θ=0 at locus D19S906). A recombination event between loci D19S905 and D19S901 defined the distal boundary and a second recombination event between loci D19S221 and D19S840 defined the proximal boundary of the genetic interval encompassing the WMS gene (12.4 cM). Interestingly, the collagen V alpha 3 gene has been assigned to this region, and appeared to be a good candidate gene by its function, but RT-PCR analysis of skin fibroblast mRNAs failed to detect any pathogenic mutations in one family. These results were confirmed by transmission electron microscopy of skin biopsies showing normal diameter and striation of collagen fibrils. Ongoing studies will hopefully lead to the identification of the disease-causing gene.

P0667. A Belgian family linked to the locus for intermediate CMT on 19p12-p13.2

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Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous disorder of the peripheral nervous system. It is characterized by progressive weakness and atrophy, initially of the peroneal muscles and later of the distal muscles of the arm. Most CMT patienst can be classified as having either CMT1 or CMT2 by use of a cut-off value of 38 m/s for the motor median nerve conduction velocity (NCV). However, in some CMT families, patients have motor median NCVs ranging from 25 to 45 m/s. This CMT type has been designated as "intermediate CMT". Recently, two loci for autosomal domiant intermediate CMT have been identified; the first locus maps to chromosome 10q24.1-q25.1 in an Italian family, the second locus maps to 19p12-p13.2 in an Australian family. Here, we performed a genome wide scan in a Belgian family with autosomal dominant CMT with 386 short tandem repeat (STR) markers of the Applied Biosystems Prism Linkage mapping set MD-10 (ABI). Fragment analysis was performed on an ABI 3700 automated sequencer. Two-point linkage analysis was performed for all STRs. Additional and flanking STRs were genotyped when LOD scores for ABI markers reached the LOD value of 1. A LOD score of 3.3 was found with markers D19S586 and D19S226, which locates the gene responsible for the CMT phenotype in the Belgian family in the region of intermediate CMT on 19p12-p13.2. However, we were not able to the delineate the 19p12-p13.2 linkage region of 16 cM.

P0668. Molecular haplotype determination using PyrosequencingTM technology

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The identification of haplotypes from a combination of single nucleotide polymorphisms (SNPs) on one chromosome is a powerful tool for genetic research. Haplotyping is usually performed statistically by computational analysis or by time consuming cloning techniques. Here we present a simple molecular approach for reliable haplotype determination on an individual basis. The procedure is based on allele-specific PCR in combination with PyrosequencingTM technology.
Allele-specific PCR primers were designed with the 3´end at the SNP position and primers specific for each allelic variant of the SNP were tested in two separate reactions. A PCR product in both reactions implied a heterozygote SNP, whereas a PCR product from just one reaction denoted the homozygote for which the allele-specific primer was complementary. A mismatch introduced in the second base from the 3´end was shown to dramatically improve the discriminatory ability between alleles. Amplified allele-specific fragments too long for analysis by Pyrosequencing technology were subdivided by nested PCR. Analysis of the SNPs using the PSQTM 96 System (Pyrosequencing AB) allowed identification of the haplotype. The genotyping after allele-specific PCR showed a typical "homozygous

pattern" of either allelic variant depending on the specificity of the allele-specific PCR primer. This procedure was used for haplotyping of fragments up to 10 kb.
Haplotype determination by the described procedure proved to be highly reliable. The results gained from Pyrosequencing technology have the benefit of being quantitative. Any amplification of a non-specific allele would therefore be detected by the system and illustrated as a peak in the pyrogramTM.

P0669. Familial Capillary Malformation Maps to Chromosome 5q

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Vascular anomalies comprise a heterogenous group of disorders the severity of which varies from life-threatening lesions to cosmetic harm. They are defects of vasculogenesis/angiogenesis and thereby provide a tool to study the mechanisms involved in these processes. Capillary malformation (CM, or "port-wine stain") is the most common vascular malformation occurring in 0,3% of newborns. CMs are small flat cutaneous lesions that consist of an increased number of ectatic capillary-like channels within papillary dermis. Vascular birthmarks, such as salmon patch, are milder variants of CM that occur up to 40% of newborns. Unlike common macular stains, the reddish coloration of CMs does not disappear, but becomes darker with advancing age. Increased incidence of lesions in first-degree relatives of CM patients and several reported familial cases suggest that genetic factors may play a role in the pathogenesis of CM. We performed a genomewide linkage analysis on 13 families with inherited CM. In non-parametric linkage analysis, statistically significant evidence of linkage (peak NPL score 6.72, p-value 0.000136) was obtained in an interval of 69 cM on 5q11-5q23. Parametric linkage analysis gave a maximum combined HLOD score of 4.84 (a-value 0.67) from the same region and the analysis using only the linked families, defined a smaller, statistically significant locus of 23 cM (LOD score 7.22). This locus contains several genes implicated in angiogenesis, such as RASA1and MEF2C. (vikkula@bchm.ucl.ac.be).

P0670. Molecular and Phenotypic Analysis of Mutations in COL8A2 in the Corneal Dystrophies

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The corneal dystrophies are a group of conditions which include Fuchs' Endothelial Dystrophy of the Cornea (FECD) and Posterior Polymorphous Dystrophy (PPCD). These conditions are characterized by a loss of corneal clarity due to an abnormally functioning endothelium (Waring et al. 1982). It is thought that this is a result of defects in neural crest differentiation (Bahn et al. 1984)
A genome-wide search of a three generation family (FECDPed1) with FECD demonstrated significant linkage with D1S2830 (Zmax = 3.72, θ = 0.0). The critical region was refined to 1p34.2 - p32. The COL8A2 gene lies within this region and encodes the α2 chain of type VIII collagen. Developmental studies have suggested a role for type VIII collagen in cell differentiation (Shuttleworth 1997) and the protein is a component of endothelial basement membrane, making COL8A2 an attractive candidate gene.
Analysis of the coding sequence of COL8A2 within FEDPed1 revealed a missense mutation (Gln455Lys) within the triple helical domain. This missense mutation was demonstrated in 2 further families with FECD and one family with PPCD. Haplotype analysis of these families suggested the presence of a common founder mutation within two out of three FECD families and the PPCD family, all of whom originate from Northern England. Haplotype analysis also suggested the presence of an identical but independent mutation a third Australian FECD family.
This is the first description of a molecular basis for the corneal endothelial dystrophies and the first association of defects in COL8A2 with human disease.

P0671. Tau negative frontal lobe dementia at 17q21: Significant finemapping of the candidate region to a 4.8cM interval

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We report the results of a genome-wide search in a 4-generation pedigree with autosomal dominant early-onset dementia (mean onset age: 64.9 years, range 53 - 79 years). In this family we previously excluded the known Alzheimer's disease genes based on linkage analysis and mutation screening of the amyloid precursor protein gene (exons 16 and 17) and the presenilin 1 and 2 genes. In addition we excluded mutations in the prion protein gene and exons 9 to 13 of the microtubule associated protein tau (*MAPT*) gene. We obtained conclusive linkage with chromosome 17q21 markers with a maximum multi-point LOD score of 5.51 at D17S951 and identified a candidate region of 4.8 cM between D17S1787 and D17S958 containing *MAPT*. Recent clinical and neuropathological follow-up of the family showed that the phenotype most closely resembled frontotemporal dementia (FTD) characterized by dense ubiquitin-positive neuronal inclusions that were tau negative. Extensive mutation analysis of *MAPT* identified 38 sequence variations in exons, introns, untranslated regions and the 5' regulatory sequence, however none were comprised within the disease haplotype. Although our findings do not entirely exclude a mutation in a yet unanalyzed region of *MAPT*, the apparent absence of *MAPT* mutations combined with the lack of tau pathology is highly suggestive for another defective gene at 17q21 responsible for FTD in this family.

P0672. An infrequent haplotype of the PCTA-1 gene, located in the susceptibility region 1q42.2-43 (PCaP), indicates association to prostate cancer

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Prostate cancer (PCa) is a complex disease with diverse genetically predisposing factors.
The underlying "defects" may range from severe rare mutations in yet unidentified genes to common variants which, in compromise to their frequencies, contribute with reduced penetrance. While high risk alleles well explain familial aggregation of PCa, observed in about 10% of all affecteds, low risk alleles may account for a higher portion of the disease, especially "sporadic" cases. Concerning a putative predisposition to PCa, we investigated the gene encoding the Prostate Carcinoma Tumor Antigen-1 (PCTA-1), mapping to 1q42.2-43, a major susceptibility region identified in a French and German genome wide search. The open reading frame of PCTA-1, which was found to be free of deleterious mutations previously, harbours several SNPs altering the amino acid sequence in four residues. In turns of a population based association study six SNPs were genotyped in 265 controls, 216 sporadic and 57 familial patients. None of the examined SNPs per se revealed association, but one out of five resulting haplotypes, which we call "C2 allele". Elevated numbers of heterozygous carriers of the C2 allele were found in sporadic (10,3% versus 3,8% in controls, p=0,0039), and familial cases (12,3%, p=0,0173). Logistic regression analyses produced relative

risks of 2,9 (CI: 1,39-6,13) and 3,6 (CI: 1,30-9,83) in the affected subgroups, respectively. This association between prostate cancer and the C2 allele may be due to the conspicuous haplotype itself, because it codes for a unique PCTA-1 protein, or could reflect linkage disequilibrium to a neighbouring susceptibility gene.

P0673. A role for Maternal MTHFR genotype in nonsyndromic cleft lip and palate.

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Nonsyndromic cleft lip and palate is a common congenital anomaly with a complex genetic component. The etiology is likely to be influenced by environmental factors. Folic acid is essential to early embryonic development and recent studies have suggested a role for “folate genes” in cleft lip. We genotyped over 200 parent-case triads for the 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism to determine whether this functional variant is responsible for nonsyndromic cleft lip and palate in affected individuals. We could find no distortion in the transmission frequency of MTHFR parental alleles tested by TDT. Examination of Hardy-Weinberg equilibrium detected an over-representation of variant MTHFR homozygotes amongst mothers of affected children when the mothers were themselves affected (odds ratio 4.61 95% CI 1.35-15.77). We postulate that these results are direct evidence of a multifactorial interaction in these families involving maternal folate status, MTHFR genotype and another locus.

P0674. Further heterogeneity in human malignant infantile osteopetrosis: evidence for a novel locus on chromosome 6q21

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Malignant infantile osteopetrosis (OMIM #259700) is a rare autosomal recessive disease. The characteristic clinical findings as osteosclerosis, hepatosplenomegaly and pancytopenia become apparent during the first months of life. Mutations have been found in the a3 subunit of the H+-ATPase and the voltage-gated chloride channel CLCN7, demonstrating that osteoclast dysfunction with inadequate bone resorption is the underlying cause of this severe disorder. In a small inbred family with malignant infantile osteopetrosis no mutation could be found in the two known osteopetrosis genes. We therefore pursued a candidate locus approach to unravel the causative gene defect in this family. We excluded homozygosity for several candidate gene loci which in mice cause severe osteopetrosis, among them c-FOS on 14q24, c-SRC on 20q11, OPGL on 13q14, and CSF-1 on 1p13. We also investigated a locus on chromosome 6q21, which is syntenic to a region on mouse chromosome 10 where the causative gene for the osteopetrotic mouse “grey-lethal” has been mapped. We detected homozygosity in a region spanning approximetly 15cM between markers D6S1717 and D6S287. In this region, there are several candidate genes, like e. g. Fyn-related-kinase (FRK), BET3, and MARCKS. We are currently conducting a systematic mutation analysis of several candidate genes in this region. In summary, we provide evidence for a novel locus for human infantile malignant osteopetrosis on chromosome 6q21 which probably represents the human ortholog of the murine “grey-lethal” locus.

P0675. Molecular Diagnosis of Haemophilia A in Bulgaria by DNA Analysis Using Polymorphisms Linked to Factor VIII Gene Locus

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Twenty - seven families affected by haemophilia A, consisting of 138 members (among these, 30 haemophilia A - affected males) were typed for polymorphisms within and outside the Factor VIII gene in order to determine carrier status of female relatives and to estimate possibilities for prenatal diagnosis when needed. Twenty of these families (74 %) did not have previous history of haemophilia A.
A panel of 8 informative DNA polymorphisms, linked to the Factor VIII gene locus was used, as follows: dinucleotide repeats in introns 13, 22 and 25; polymorphisms in presence/abcense of restriction sites in introns 18, 19 and 22; a SSCA polymorphism in intron 7 and a tandem repeat in the DXS52 locus, located outside of the Factor VIII gene locus.
Twenty five (82 %) families showed informativity at at least one marker locus. Only 2 families (8 %) were uninformative at all the markers used.
Pedigree data identified 29 women at reproductive age (mothers of affected boys not included) which were of high risk of having a haemophilia A - affected boy. DNA analysis allowed determination of carrier status in 25 women (83 %), of those 16 women were identified to be haemophilia A carriers and 9 noncarriers.
Ten prenatal diagnoses were performed by analysis of DNA polymorphisms. Seven of these pregnancies were carried to term, producing 5 girls and 2 healthy boys. One male fetus was diagnosed to be at high risk to be affected by haemophilia A, nevertheless, parents did not opt for an abortion.

P0676. DNA Variability of Human Genes

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We have investigated the level of DNA-based variation (both SNPS and haplotypes) for over 5,400 human genes. In addition, we have characterized how this variation is distributed in a number of biologically and clinically important ways. First, we have determined how SNPs are distributed in human genes: where they occur relative to various functional regions; levels of variability of human SNPs; pattern of the molecular sequence of SNPs; and how these compare to the corresponding sequence of a chimpanzee. Second, we have determined how these aspects of SNP distribution vary among four human population samples. All genes were sequenced on DNA obtained from 82 unrelated individuals: 20 African-Americans, 20 East Asians, 21 European-Americans, 18 Hispanic-Latinos and 3 Native Americans. In particular, we looked at patterns of SNP and haplotype sharing among the four larger population samples. Third, we have determined the patterns of linkage disequilibrium among SNPs, which of course determines the haplotype variability of each gene. This pattern also varies substantially among populations. In order to connect important clinical variability (e.g., genetic disease or susceptibility, variable drug response) to the DNA variability of human genes, an understanding of these patterns of variability within and among human genes is a fundamental prerequisite.

P0677. Evidence for linkage of aggressive prostate cancer to chromosome 7q32 in German prostate cancer families

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Chromosome 7q32 has been suggested to contain genes that influence the progression of prostate cancer from latent to invasive disease by Witte et al. [AJHG 67:92-99,2000]. This locus did not show up in previous genome wide scans and emerged by QTL analysis in sib pairs. We looked for linkage of prostate cancer aggressiveness to chromosome 7q by stratification in 108 German prostate cancer families according to lymph node affection and grading of the tumor. A panel of 8 polymorphic markers (PE Applied Biosystems) on 7q was used. We found no evidence of linkage between a prostate cancer susceptibility locus and markers on chromosome 7q in the complete set of 108 families. However, the subset of families with aggressive prostate cancer (positive lymph nodes in one family member or two cases of GIII tumors) had an NPL of 1.50 (p = 0.06). Most evidence for linkage came from a few families with aggressive and late onset disease, mean age of onset >65 years and NPL of 2.56 (p=0.01). D7S640, the marker with the higest NPL in our analysis, is located exactly between the markers that gave the strongest signals in the study of Witte et al. [AJHG 67: 92-99,2000]. In the German population, chromosme 7q32 is linked to a type of prostate cancer that is characterized by a late onset and an aggressive course of the disease.

P0678. Confirmation of Genetic Homogeneity of Syndactyly Type 1 in an Iranian Family

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Syndactyly type 1 (SD1) is the most common type of syndactyly, inherited in an autosomal dominant fashion, and characterized by complete or partial webbings between the third and fourth fingers and/or between the second and third toes. We recently encountered an Iranian family in which 33 members in six generations were affected with SD1. As a locus of SD1 in a German family has recently been assigned to chromosome 2q34-q36, we performed a linkage analysis of the Iranian SD1 in order to know whether the disorder is genetically homogeneous. With the analysis on 15 affected and 16 unaffected persons using dinucleotide repeat polymorphisms as markers, we mapped the SD1 locus to 2q34-q36 with a maximum LOD score of 6.92 at a recombination fraction q = 0.00 (penetrance = 1.00) for the D2S2179 locus. The result not only confirmed the gene assignment but also suggests genetic homogeneity of the disease.

P0679. Identification of athe locus for ichthyosis-prematurity syndrome on chromosome 9.

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Autosomal recessive congenital ichthyosis (ARCI) is a heterogeneous group of skin disorders. Ichthyosis-Prematurity Syndrome (IPS), is a rare form of ARCI with a relatively high prevalence in the Norwegian population.
Key features are thick caseous desquamating epidermis and complicated pregnancies probably due to polyhydramnion and an opaque amnion fluid caused by the shedding of large amount of epidermally derived cells. This results in premature birth of the affected child.
Thirteen families with at least one affected member and one healthy or affected sibling were identified. Altogether, the 13 families included 17 affected members and 13 healthy siblings. All families are related to a defined region in middle-Norway and Sweden.
A genome-wide linkage analysis gave indication for linkage to chromosome 9q, and further analysis resulted in a maximum cumulative lod-score of 3.73 in the 9q34 region.
Haplotype analysis of meiotic recombination events refined the genetic interval to a 9 cM region between markers D9S250 and D9S63. This restricts the IPS locus to a physical distance of 8 Mb in the chromosome 9q33.3-q34.13 region.

P0680. Genomic mapping of a fourth gene involved in Familial Hypercholesterolemia

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Autosomal Dominant Hypercholesterolemia (ADH) is one of the most common hereditary diseases, characterized by a selective increase of LDL particles, giving rise to premature mortality from cardiovascular complications. ADH results from molecular defects in the LDLR gene (Familial Hypercholesterolemia), the APOB gene (Familial ligand-Defective apolipoprotein B-100) and the FH3 gene (1p32-p34.1). We identified a large French ADH family (HC6) in which the involvement of these 3 genes had been excluded suggesting the existence of a fourth locus (FH4). We undertook the identification of the FH4 locus using linkage analysis in family HC6 by a candidate region approach. After the exclusion of 30 candidates, we undertook a whole genome approach with 220 new microsatellite marker. Linkage was obtained with a LOD score of 3.86 (q=0) and confirmed by a multipoint LOD score analysis. Suggestive linkage was also obtained for 4 other nonLDLR/nonAPOB/nonFH3 families conforting the localization of the FH4 locus in this genomic region. Finally, a critical region of 1.9 cM was defined which contains the FH4 gene. We constructed a 3.8 Mb physical map covering our genetic region and identified 82 genes, that we are testing by direct sequencing in collaboration with the Genoscope.

P0681. Chromosome 20p is linked to prostate cancer susceptibility in the German population

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Chromosome 20q13 has been suggested to harbour a prostate cancer (PC) susceptibility locus in families that were characterized by a low number of affected familiy members, late onset disease and no male-to-male transmission. As this epidemiologic profile is characteristic for many German PC families we performed linkage analysis using 7 markers on chromosme 20. There was no evidence for linkage in the whole sample of 108 PC families and in the subsets with mean age of onset < or > 66 years or with the number of affecteds/family < or > 3 as criteria for stratification. In a previous study we identified a subset of families that were linked to chromosome Xq27-28 (NPL > 1) but at the same time had an affected individual in the paternal line of their pedigree. In this subset of 26 families with the conflicting characteristics of male-to-male transmission and X chromosomal allele sharing, multipoint parametric linkage analysis (Genhunter 1.3) showed a maximum LOD score of 4.10 (p=0.001) at D20S112. This may indicate interaction of two genetic determinants of PC susceptibility. Marker D20S112 is localized on 20p11.2-12 and is distinct from the susceptibility locus (D20S887) suggested in the initial study on chromosome 20q.

P0682. Linkage disequilibrium mapping of the HLA-linked *MYAS1* locus for autoimmune myasthenia gravis.

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Myasthenia gravis (MG) is an autoimmune disorder of the neuromuscular junction characterized by production of auto-antibodies against muscle acetylcholine receptors (AChR). Recently, we demonstrated linkage and association of the extended HLA-DR3 haplotype with the form of MG associated with thymus hyperplasia and high auto-antibodies titers, defining the *MYAS1* locus. In the present study, given the strong linkage disequilibrium across the DR3 haplotype, we sought to refine the localization of the *MYAS1* locus by combining TDT and ANOVA of AChR antibody titers. A panel of fourteen microsatellites and six SNPs, evenly spaced across a 2.5 Mb region between the DRB1 gene and the D6S265 microsatellite, ~100 kb centromeric to HLA-A, was genotyped in 717 MG patients and, for 228 of them, in their relatives. Using single-locus TDT, the strongest association was observed with the TNFd*1 allele, in the class III region ($P=1x10^{-5}$). No association was observed with D6S265, which therefore defines the telomeric boundary of the *MYAS1* interval. Two-locus TDT bearing on ancestrally-recombinant haplotypes reduced the *MYAS1* interval to 700 kb excluding both DRB1 (P=0.008) and C2.4.5 (P=0.007) which is located between HLA-B and -A. ANOVA of anti-AChR antibody titers further narrowed the *MYAS1* interval. Variance was best explained by MH*169 ($P=7x10^{-4}$) and TNFd*1 ($P=2.6x10^{-3}$) and by their combination ($P<1x10^{-12}$). A primary region where to look for the *MYAS1* gene may be therefore a 40 kb DNA segment limited by these two markers. It currently includes four genes which are under active investigation.

P0683. Refinement of a locus for a distinct syndrome of autosomal dominant cleft lip and palate to 2q35

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Orofacial clefting is a common craniofacial anomaly that occurs in many multiple congenital anomaly (MCA) syndromes. Kumar et al (1996) reported a dominant MCA with cleft lip and palate and characteristic facies in two families of Caucasian origin. The phenotype in these families overlaps with both Hypertelorism, Microtia, clefting syndrome (HMC) and Fronto-nasal dysplasia but we believe this dominant cleft lip syndrome may be recognisable as a distinct entity. We have carried out a whole genome scan in these two families and identified co-segregation to a locus on 2q35. For the larger family we have used additional family members to refine this locus to a region of 10 Mb across a haplotype of four markers which co-segregate in 6 affected individual and one apparently unaffected individual. We have excluded the candidate genes DLX1 and DLX2

by sequencing and fine mapping. All other regions of the genome have been excluded except for the short arm of the X chromosome and work is currently underway to confirm exclusion at this locus. These results demonstrate a major dominant cleft lip locus with high but possible incomplete penetrance.

P0684. Genetic analysis of two unrelated Italian Families with non specific X-mental retardation

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X-linked non specific mental retardation (MRX) accounts for ~ 25% of mental retardation in males. Despite this high frequency, little is known about the molecular defects underlying this disorder, mainly because of the clinical and genetic heterogeneity which is evident from linkage studies. A wide variety of MRX loci have been mapped on X chromosome. At least 8 MRX genes have been identified , but each accounts for only 0.5-1.0% of MRX cases. Here we report two MRX families. The first family has nine males in two generations with classic X-linked inheritance of variable degree of non specific mental retardation.We have performed on this family a two point linkage analysis that shows tight linkage for marker GATA72E05 with Lod Score of 3.14 at q=0.00. Two point linkage interval corresponds to roughly 23 cM in the pericentromeric region of X chromosome. According to linkage data and their functional characteristic, we are performing the mutational screening of some genes in this region to prove their involvment in this patology.
The second family is composed by eight males in three generation characterized by a mild to severe X-linked mental retardation. Previous analysis linked this family in Xq28 between marker DXS1073 and F8c (Lod Score=2.71 at q=0.00). We have performed mutational analysis for 10 candidate genes present in this region by sequencing and RT-PCR analysis and we have found some known and unknown polymorphisms. We are now looking for other candidate genes and we are performing further analysis to esclude genomic rearrangements.

P0685. Whole Genome SNP Scans: what is currently possible and affordable

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SEQUENOM has worked with Incyte Pharmaceuticals and Glaxo Smith Klein to develop a set of validated SNP assay portfolios that altogether number about 200,000 polymorphic SNPs. These assays can be run on pooled DNA samples to generate accurate estimates of allele frequencies in populations of interest. Allele frequency differences in phenotypically-stratified populations can reveal genes with strong associations to phenotypes. Alternatively SNPs can be genotyped on individual DNAs and allele and genotype-phenotype correlations done subsequently *in silico*. The relative advantages and costs of the two procedures will be compared. For individual genotyping multiplexed sets of SNP assays can cut costs considerably. Progress in developing a multiplexed genome scanning set will be described. Using these methods individually, and in combination, a number of interesting gene associations to complex disease phenotypes have been discovered. The potential importance and utility of some of these associations will be demonstrated.

P 15. Mental Retardation

P0686. Challenges that arise from a routine MECP2 mutation testing service.

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An initial surge of demand for MECP2 mutation analysis has settled into a steady-state pattern. We have screened for mutations using bi-directional sequencing. Among 292 samples referred, results have fallen into four major categories: pathogenic nucleotide variant (74); polymorphic variant (14); variant, pathogenicity indeterminate (13); with the remainder being wildtype. Among the wildtype results, we are aware of 13 cases where the clinical diagnosis is Rett syndrome and another 10 in whom the diagnosis is strongly suspected. When an indeterminate nucleotide variant is detected, follow-up analysis is recommended. This may include evaluation of parental samples, RNA and X chromosome inactivation (XCI) studies. Three cases from the indeterminate group have been selected to reveal the complexities that can occur, which have ranged from assessing novel missense and silent nucleotide variants that have not been reported previously among more than 1400 published mutations; evaluating non-coding variants that are highly conserved; and interpreting cDNA findings in the face of skewed XCI. It is evident that laboratories that offer a comprehensive mutation testing service for MECP2 and other genes on the X chromosome must be sufficiently resourced to meet the full range of challenges that can arise.

P0687. Rett syndrome in females and in a male

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Rett syndrome is an neurodevelopmental disorder characterised by autism, dementia, ataxia and loss of purposeful hand use.
The exclusive involvement of females was explained by X- linked dominant inheritance with lethality in the hemizygous males. The responsible gene has been identified in the Xq28 region and encodes the methyl-CpG binding protein 2 (MeCP2). A non fatal Rett syndrome in male has been published in the year 2000. The authors confirmed the somatic mutation in MeCP2 gene.
We have a small cohort of 8 girls with Rett syndrome. The mutation analysis revealed different mutations in MeCP2 gene (R294X, K135E, T158M, 1069delAGC). Recently we examined a 23 year male with classical features of Rett syndrome. The normal perinatal period was followed by a period of regression, loss of acquired skills, ataxia, gait disturbance and deterioration of the brain functions. The numerical aberration of the X chromosome was excluded, the man has a normal karyotype 46,XY. We expected that the mosaic mutation using the methods based on heteroduplex analysis and sequencing will be detected.

P0688. MECP2 mutations in males; Report of a case with Prader-Willi-like phenotype

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Heterozygous mutations in the X-linked *MECP*2 gene have first been described in Rett syndrome (RS), a progressive neurologic developmental disorder, occurring almost exclusively in females. Diagnostic criteria for RS were established by Hagberg *et al*.
Recent studies indicate that *MECP*2 mutations are not necessarily prenathally lethal in males, but are the cause of lethal congenital encephalopathy and even of non-fatal congenital encephalopathy. Of the latter group, some patients have clinical features suggestive of the Angelman syndrome (AS), others are reported with nonspecific mental retardation (MRX) only. The clinical and molecular features of an additional male patient with a *de novo MECP*2 mutation are presented and compared to previous reported cases.

P0689. Refined molecular characterisation of a *de novo* t(5;18)(q33;q12) associated with Rett-like syndrome and autism

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Mutations in the MECP2 gene cause the severe neurodevelopmental disorder called Rett Syndrome. Children afflicted with Rett Syndrome often also exhibit autistic-like behaviours. Beside MECP2 mutations, several cases of structural abnormalities of autosomes associated with Rett Syndrome are known. Here we report on a 10-year-old boy with a reciprocal 5/18 translocation. His clinical manifestations are a combination of mild dysmorphic features and those of Rett Syndrome and autism. Due to several cases of autism in patients with 18q deletions, our primary goal was do narrow down the breakpoint region on chromosome 18. Fluorescence in situ hybridisation experiments using various region specific YAC- and BAC clones led to split signals in a 200 kb sequenced BAC clone indicating that

the corresponding DNA insert is spanning the breakpoint region. Subsequent Southern blot analysis with probes derived from the spanning BAC clone was performed to isolate a junction fragment. Sequence analysis of the junction fragment will help to find candidate genes for the neuropsychiatric features seen in our patient.

P0690. Genotype / Phenotype Correlations In A Large Series Of Rett Syndrome Patients

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Mutations in the human Xq28-linked MECP2 gene are responsible for 70 to 80% of Rett syndrome cases and also for atypical clinical presentations.
We are presenting the results of our studies in a serie of 64 female cases in which we looked for mutations in the MECP2 gene. These patients were divided into three groups :
- Group I (23 patients) with Rett syndrome diagnosed in our multidisciplinary consultation.
- Group II (28 patients) referred by external collaborators for a suspicion of Rett syndrome
- Group III (17 patients) from our center exhibiting a severe encephalopathy with autistic behaviour.
In the first group, 21 mutations were identified ; in the second, 8 mutations and zero in the third group. These results confirm that the vast majority of mutations in the MECP2 gene lead to a classical Rett syndrome phenotype. They also show that a large proportion of girls with a severe mental handicap are not found to be associated with a MECP2 mutation on the contrary to what was recently proposed in the litterature.
In addition, we did not find any genotype-phenotype correlations for the Rett syndrome patients taking into account the mutation type, the functional domain affected by the mutation and the X-chromosome inactivation status of these patients except for the mutation involving the C-terminal domain associated with a « forme frustre » of the disease. It is thus likely that a number of not yet identified factors are responsible for the clinical variability of this syndrome.

P0691. Fragile X syndrome : clinical and behavioral study.

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The present study included 25 boys with mental retardation of unknown etiology and their mothers. Each boy manifested, at least, two of the following features: positive family history of mental retardation, long face, large ears, hyperextensible finger joints and bulbous halluces. Patients were assessed for 18 physical features and 23 behavioral features. Reverse transcriptase-polymerase chain reaction (RT-PCR) amplifies across the two KH domain regions specific for FMR1 gene. Diagnosis of patients was absolutely dependent on the detection of absent FMR1 transcriped mRNA. This approach successfully diagnosed fifteen boys (60%) from 11 families as fragile positive and ten boys (40%) from 8 families as fragile negative. Multiple regression analysis revealed 11 features (4 physical and 7 behavioral) with high predictive value for fragile X syndrome. The frequency of speech defect was significantly increased in boys with fragile X syndrome. Delayed language, numerical and time concepts, domestic activities and directive behavior were significantly delayed in those boys. Eysenk personality questionnaire, Beck depression inventory and parental attitude questionnaire were applied to all mothers and revealed increased psychoticism, neuroticism, criminality and various degrees of depression in mothers of fragile X boys. In conclusion, the combination of physical and behavioral traits is helpful in suggesting the diagnosis of fragile X syndrome. The preselection 5-criteria device proved suitable for detection of fragile X candidates, thereby improve the cost-effectiveness of fragile X molecular testing. We propose application of this device in clinical pediatric practice for early detection of fragile X syndrome in young children.

P0692. Effect of premutation in the FMR1 gene on cognitive and physical phenotype in fragile X assessed by pedigree analysis.

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The effect of premutation status in the FMR1 gene on cognitive and physical measures in fragile X males and females was investigated using a robust modification of the maximum likelihood estimators for pedigree data. This approach is much more powerful than standard statistics because it allows for testing model assumptions, such as concerning the effect of explanatory variables, and for reducing variability in the data by down-weighting unusual observations, and adjusting for intra-familiar variation. It also allows for estimation of heritability of complex traits. The data from extended 110 fragile X families (including 185 fragile X subjects and 110 normal relatives) were analyzed. Fragile X status was determined by the number CGG repeats in the FMR1 gene. Physical phenotype was represented by trunk, limbs, head and face measures, and neuropsychological phenotype, by the cognitive (Wechsler), and executive function measures. Evidence is presented for strong phenotypic (neuropsychological) involvement in premutation carriers, especially in males, which predominantly affects performance skills and executive cognitive functioning. The effect of premutation status on physical phenotype was less evident and concerned only some facial measures in both sexes, and the measure of joints' laxity in females. Heritability ranged from the highest (88%-90%) for physical measures, to the lowest (10%) for some executive function measures.

P0693. Should all girls learning disabled or mildly retarded be screened for FMR1 mutation?

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Fragile X syndrome (FXS) is the most common inherited cause of mental retardation, with a prevalence of about 1/4.000-5.000 in males. Therefore many clinicians must deal with fragile X individuals in their daily routine, but many of them still don't recognise this syndrome. This problem is greater in women, in whom the prevalence is theoretically the same as in males, but due to the fact that at least 50% of the fully mutated women are normal, the clinical prevalence in females is in fact much lower, about 1/10.000.
Our laboratory has been working on FXS molecular testing since 1991, receiving the majority of requests for FMR1 testing from Northern Spain. Since our first description of a girl with the FRAXA full mutation with no history of mental retardation in her family (Tejada et al., 1998), we have been recommending to test women for FXS. Moreover, in cooperation with "The Fragile X Association" we have been lately developing an information program to different professionals: pediatricians, psychologists, teachers, etc.
Here we present the molecular results of the 2001-year and compared them with the 10 previous years. In this later year we have studied 38 case index females and found 3 new non-related cases of FXS (3/38= 7,89%) without affected males in their families. We also report the initial symptoms, behaviour features, physical description and mental status of these 3 girls (aged 5, 3 and 5 years respectively), to add new data on the knowledge of fragile X syndrome in women.

P0694. Fmr-1 full mutation in leukocytes of a female not affected by X-Fragile syndrome.

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A 35 years old woman was evaluated for FMR-1 mutation because her children (a male and a female) were found to be FMR-1 full mutation (CGG > 200 repeats). The laboratory tests performed to investigate the X fragile syndrome were: 1) chromosomal analysis 2) PCR of blood sample 3) Southern Blot method after treating DNA

from leukocytes with Eag and EcoRI restriction enzymes. Results: 1) normal 46, XX caryotipe in blood nucleated cells without fragile sites detected, 2) a single faint band of PCR product following FMR-1 gene amplification, 3) the absence of 2.8 Kb band corresponding to the unmethylated FMR-1 allele, accompanied by a smear in correspondence of the methylated allele. To interpret these findings we hypotize that in blood cells of this women the ratio between FMR-1 full mutation and FMR-1 normal allele might be in favour of the abnormal allele. This could be supported by the not clear result on PCR. The peculiarity of this young woman is the absence of mental retardation and other classical abnormality typical of the X fragile syndrome, so that we can suppose a rare form of FMR-1 abnormality involving at least her blood leucocytes. Further investigations about other forms of tissue mosaicism are in progress.

P0695. Genetic-Diagnostic Survey of 3570 Children with Mental Retardation

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This study was conducted on 3570 subjects who were referred to our outpatient clinic from July 1, 1985 to December 31.1998 for evaluation of mental retardation (MR) Medical records and photographic documentation were reviewed in all. History, physical and neurological examinations, chromosomal analysis, molecular studies, skeletal surveys; cranial imaging studies, electroencephalography and metabolic screening test were performed as indicated.
Patients were classified into two groups as patients who have isolated MR only, and MR with multiple congenital abnormalities (MKA+MR).
MR group (n: 582) consisted of known monogenic syndromes (n: 90), enzyme deficiencies (n: 112), patients of unknown etiology with MR (n: 380),
MKA+MR group (n: 2988) consisted of chromosome abnormalities (n: 1384)., recognizable syndromes (n: 55), known monogenic disease (203), structural central nervous system abnormalities (n: 540) and unclassified patient with MKA+MR (n: 806)
Out of 582 patients with MR and 2988 patients with MKA+MR etiology were noticed into 202(34.7 %) and2182 (72.02 %) respectively.
The results were compared with the similar studies in literature.

P0696. High occurrence of Brachydactyly-Mental Retardation syndrome among mentally retarded subjects in Italy

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Background Brachydactyly-Mental Retardation syndrome (BDMRS), MIM 600430, also defined as Albright Hereditary Osteodystrophy-like (AHO3) syndrome, is associated to distal deletion on 2q37 region. This association was first described independently by Phelan et al and Wilson et al (1995).
Results MultiFISH based screening for subtelomeric chromosome defects (Cytocell) was performed on 250 mentally retarded patients. The observed frequency of subtle chromosomal abnormalities was 6% (15/250). Among the fifteen subjects carrying chromosomal rearrangements six showed 2q37 de novo deletions (2.4%) associated to BDMRS. Since the probe included in the kit still detects 2q37 telomeric polymorphism (Macina et al, 1994; Knight et al, 1997), for all patients the 2q37 deletion was mapped by microsatellites and FISH analysis using genomic probes (PAC and BAC) encompassing the chromosomal region. Parental origin was determined by microsatellites analysis. The occurrence of the length polymorphism at 2q37 was also found in 17 subjects (7%). In two of them the polymorphism was present in compound heterozygosity with the BDMRS-associated deletion.
Conclusion Advancement of FISH strategies allowed to screen large series of mentally retarded patients and consequently improve the frequency definition of some recurrent chromosomal aberrations escaping from conventional karyotype. Among previously reported patients (Phelan et al and Wilson et al, 1995) with 2q37 deletion, only one of nine showed a normal karyotype. The group of patients presented here carried submicroscopic deletions allowing the evidence that BDMRS is an emerging clinical grouping. The presence of chromosomal polymorphisms is under investigation to verify a genetic predisposition to 2q37 rearrangements.

P0697. X-linked Lissencephaly with Absent Corpus Callosum and Ambiguous Genitalia (XLAG). Clinical, MRI and Neuro-Pathological Findings.

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X-linked Lissencephaly with absent corpus callosum and ambiguous Genitalia (XLAG) is a newly recognized syndrome responsible for a severe neurological disorder of neonatal onset in boys. Based upon the observations of three new cases, we confirm the phenotype in affected boys, we describe additional MRI findings, we report the neuropathological data and, we show that carrier females may exhibit neurological and MRI abnormalities. In affected boys, consistent clinical features of XLAG are intractable epilepsy of neonatal onset, severe hypotonia, poor responsiveness, genital abnormalities and early death. On MRI, a gyration defect consisting of anterior pachygyria and posterior agyria with a moderately thickened brain cortex, dysplastic basal ganglia and complete agenesis of the corpus callosum are consistently found. Neuropathological examination of the brain shows a tri-layered cortex containing exclusively pyramidal neurons, a neuronal migration defect, a disorganization of the basal ganglia and a gliotic and spongy white matter. Finally, females related to affected boys may have mental retardation and epilepsy, and often display agenesis of the corpus callosum. These findings expand the phenotype of XLAG, may help in the detection of carrier females in affected families and give arguments for a semidominant X-linked mode of inheritance.

P0698. Long Term Follow-up Of A Girl With Oro-facio-digital Syndrome Type I Due To A Mutation In The Ofdi Gene

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In 1954 Papillon-Léage and Psaume described a dominant, X-linked condition which they named oro-facio-digital (OFD). This condition was split in at least 9 syndromes, the more common being OFDI. We report a girl with OFDI syndrome followed-up during 24 years.
The parents were unaffected. An older brother had hydrocephaly, and mental retardation. A younger sister is unaffected.
The proband was examined for the first time when she was 3 months old for median cleft lip and cleft palate, lingual frenula and hypoplasia of the maxillary and the mandible. She had clinodactyly of the 5th fingers, shortening of fingers and toes 3, 4 and 5 and syndactyly. She was operated on several times.
At 19 years of age renal insufficiency appeared. Renal transplantation was performed.
A mutation, an insertion of a G leading to a frameshift in the OFDI gene encoding a protein containing coiled-coil α-helical domains was identified in the patient who was asking for prenatal diagnosis when she was 24 years old.
Associated malformations of the OFDI syndrome are cerebral, and renal. There was no developmental delay and no cerebral malformations in this patient. There were multicystic kidneys with renal insufficiency leading to renal transplantation. The results were good.
In conclusion a girl with OFD type I syndrome was followed up during 24 years. A renal transplantation was performed when she was 20 years old. A frameshift mutation of the OFDI gene was identified.

P0699. X-linked mental retardation with cerebellar hypoplasia

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We report a three genration family including 4 boys aged 3 to 17 years presenting with moderate mental retardation, abnormal facial appearance and brain anomalies. Mean age at walking was 30 months. Language was severely delayed and reading was not acquired in the eldest ones. All had tall stature above 2SD. Facial appearance was similar with macrocephaly, deeply-set eyes and and prominent chin. Brain MRI showed the same findings in all affected boys: supratentorially, mild to large dilatation of the ventricles was noted without cortical dysplasia. On of the boys had vetriculo-peritoneal shunt because of marked dilatation of the third and lateral ventricles. The most prominent abnormalities were seen in the posterior fossa: the cerebellar vermis was hypoplastic with various degrees of Dandy-Walker complex. The two obligate carrier females demontrated mild mental retardation and tall stature. Brain MRI showed diffuse cerbral atrophy in both and a slight supratentorial expansion of the cisterna magna through a posterior hiatus of the tentorium in one.
We compared the phenotype of these patients with the one described in previously reported families with X-linked mental retardation with cerebellar abnormalities. In most of these reports, the clinical picture was characterized by progressive cerebellar atrophy and clinical ataxia, which is very different from the pure mental retardation with congenital cerebellar hypoplasia seen in our patients.
Assuming X-linked inheritance, linkage analysis using 32 highly polymorphic parkers evenly distributed along the X chromosome was performed. There was no recombination between markers DXS1039 (Xp11.23) and DXS1047 (Xq25).

P0700. Clinical investigation and candidate gene screen of families mapped to the Partington syndrome region in Xp22.1.

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Partington et al. (1988) described a three-generation family (MRXS1, MIM *309510, PRTS) with a syndromic form of X-linked mental retardation (XLMR). The clinical features in 10 affected males included mild to moderate mental retardation, dystonic movements of the hands, dysarthria and seizures. Through linkage analysis a maximum LOD-score of 3.1 was obtained at marker DXS989 with flanking markers DXS365 and DXS28 (Xp22.1). The PRTS region comprises about 10 megabase and includes 9 novel transcripts and 13 known genes that are expressed in brain and muscle. The PRTS is a rare and specific clinical entity. So far, no patients other than the original family have been described. We present two brothers with PRTS and three males of a two-generation family with non-specific XLMR (MRX36), who after clinical reinvestigation show PRTS features. Neurological features in the affected males include moderate mental retardation, dysarthria, facial muscle weakness, severe dysdiadochokinesis, slow dystonic movements and mild spasticity of the hands without ataxia or spasticity of the legs. The symptoms are non-progressive, extra-pyramidal and without cerebellar involvement. We try to further delineate the PRTS phenotype and based on recent findings of Dr. J. Gecz and Dr J. Chelly, we will present data on a candidate gene screen performed on the two brothers with PRTS, MRX36, a family with West syndrome and on a selected group of MR male-patients.

P0701. X-linked lissencephaly with absent corpus callosum and ambiguous genitalia (XLAG) : a new family with severe expression in a girl

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X-linked lissencephaly with absent corpus callosum and ambiguous genitalia (XLAG) is one of the distinct malformation syndrome associated with lissencephaly. Only 9 male patients were reported since the first description by Berry-Kravis in 1994. All were severely affected with intractable epilepsy from birth, profound mental retardation, temperature instability, growth retardation and ambiguous genitalia. Neuroradiologic findings were lissencephaly with a posterior-to anterior gradient, intermediate increase in cortical thickness, corpus callosum agenesis. Recently, Bonneau et al. described female carriers phenotype: all carriers (5/5) had partial or complete corpus callosum agenesis with no or mild mental retardation. None had severe mental retardation nor seizures. We report a new family. The propositus, a male, presented at birth with the classical type of the disease and died at day 10. His sister had seizures at day 2. At 3 years, she had profound mental retardation and severe epilepsy. RMI showed corpus callosum agenesis, posterior agyria-pachygyria and abnormal white matter. Karyotype was normal 46 XX. No mutation was detected in XLIS gene. X-inactivation study is on progress. The mother had normal intelligence but RMI showed partial corpus callosum agenesis. This observation confirm the frequency of corpus callosum agenesis in XLAG carriers. In contrast, a severe phenotype was never observed in female carriers and skewed X-inactivation is supposed to be responsible for severe expression in this girl.

P0702. Report of 2 new FRAXE families detected by FRAXA and FRAXE post-natal screening

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FRAXA is one of the most common cause of mental retardation for boys, FRAXE is rarely reported. From 1997 to 2001, 857 families were screened for FRAXA and FRAXE amplification. The study concerned 581 boys and 276 girls showing non-explained developmental delay. CGG and GCC repeat amplifications have been studied for respectively FMR1 and FMR2 genes. Through this screening 11 new FRAXA families and 2 new FRAXE families were detected using respectively Pergolizzi and Knight's modified PCR protocole (Cell 1993) and chemiluminescence for agarose gel revelation.
The first FRAXE proposant was an 8 years old boy affected by mental retardation, stuttering and frontal headache, constipation, cryptorchidy and corpus callosus agenesis.He had no facial dysmorphism but strabismus. WISC-R showed: 54 (verbal : 66, performance : 51) , he was not efficient in mathematics.
PCR revealed an abnormal amplification over 350 GCC repeats for him, 115 repeats for his mother and 71 repeats for his grand-mother.
The second FRAXE proposant was a 14 years old boy, with long face and nose, a mouth finely drawn, small ears, photosensible seizures, and headache. He was very anxious, and had difficulties in mathematics. PCR revealed an abnormal amplification over 320 GCC repeats for him, 203 repeats for his healthy sister, 121 for his mother, and 50 repeats for his grand-mother. four other men and women had amplification over 83 repeats in this family. A prenatal diagnosis was realised for his sister and only the sexe status of the female foetus was announced .

P0703. Coffin-Lowry's syndrome: About a familial case

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Coffin-Lowry's syndrome is individualized in 1975 by Temtamy from 2 descriptions princeps of Coffin and Lowry. It is an X-linked semi- dominant inherited disorder, mental delay is deeper at the boy's, the gene is localized and identified on the short arm of the X chromosome, in Xp22-2, it codes for a protein kinase RSK2. The incidence of this syndrome is to be about 1 per 50-100 000 males/ year.
We report the observation of a mother and its daughter presenting a mental retardation, an obesity, a facial dysmorphy, a digital abnormalities and a deformations of the skeleton, evoking Coffin-Lowry's syndrome .The investigates genetic find similar cases in the family. We begin in this work a comparative study with the data of the literature, we discuss the differential diagnosis, and we shall insist on the interest of the molecular study in a purpose of antenatal diagnosis.

P0704. Prospective Evaluation Of Audio-Visual Function In Down Syndrome Infants

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There is limited knowledge of early development of visual and auditory function in Down syndrome.

In this study audio-visual function of 35 Down syndrome infants (0-6 months old) were analyzed and followed prospectively 3-6 months later.

In assessment of auditory function, other than the 7 infants with chronic otitis media, brain-stem auditory evoked potential (BAEP) testing was applied to the remaining 28 infants. Compared with age-matched 62 normal children the major anomalies were significantly elevated response threshold (%39 hearing loss) and poorly differentiated wave I. Latency of wave V and I-III, III-V and I-V interpeak intervals were shorter but were within the 2 standart deviation. As a developmental change, follow-up testing showed that interpeak intervals continued to shorten with age.

In assessment of visual function, 35 Down syndrome infants (0-6 months old) were evaluated with visual examination and flash visual evoked potentials (FVEP) testing. Compared with age-matched 36 normal infants, latency of N2 and P2 were longer ($p< 0.05$) and amplitude of N2 and P2 were smaller in Down syndrome patients ($p< 0.05$).

The results of this study reveal that in Down syndrome the development of peripheral hearing and vision is delayed and also abnormal. BAEP and VEP testing are proposed to assess the development and detecting the problems of hearing and vision in Down syndrome infants. These tests should be repeated between periods of 6-12 months even if the results of initial tests are normal.

P0705. Cognitive and Behavioral Phenotype of Children with Some Common Dysmorphic Syndromes

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Testing the cognitive capacity is a relatively new approach when examining patients having genetic diseases. These diseases often tend to be diagnostic problems due to their wide variety. That renders the precise estimation of the cognitive and behavioral phenotype a great importance.

Aim: to study cognitive and behavioral characteristics of patients with dysmorphic syndromes most often met in clinical practice (Down, Frigali X, Prader-Willi, Williams-Beuren, Noonan and Cornelia de Lange) and to define the specific cognitive and behavioral phenotypes.

Results and discussion: The different dismorphic syndromes manifest cognitive capacity deviations of different severety. IQ level in an individual patient changes with age in different ways. In our experience patients with Down, FRAX-A and CdeL syndromes show an intellectual decline with age, as other authors have observed, while patients having PWS, WBS and NS manifest an unequivocal tendency to imrove their cognitive skills. The most maladaptive behavior is shown by patients with CdeL and FRAX-A. The relationship between intellectual functioning and adaptive skills is in inverse proportion - for example for CdeL syndrome there are low IQ values and a large number of psychopathological behavioral symptoms hampering adaptability, while for NS there are comparatively high IQ values and negligible behavioral deviations.

Conclusions: The patients with some dismorphic syndromes manifest more or less specific psychological profiles of cognitive and behavioral characteristics. These profiles may be successfully used both in support of diagnostic process and as a basis for adequate medical and psychological intervention and counselling of parents.

P0706. Asperger syndrome and severe language impairment in two siblings, as the type and countertype of an unbalanced cryptic familial 22qter rearrangement.

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Numerous cytogenetical and molecular evidences suggest that terminal 22q region is involved in language development. Indeed, many 22qter cryptic deletions were recently reported from patients harbouring a mild mental retardation, absent or few dysmorphic traits, frequent behavioural problems and severe speech impairment as a constant feature. We report clinical and molecular data from a family where a terminal 22q deletion was identified. The proband, a 8 years old girl suffered of a global development delay with a patent discrepancy between development of speech and of the other areas. This deletion was inherited from a paternal balanced translocation between the terminal end of a 22q arm and the p arm satellites of a chromosome 14. The karyotype of her father was 46,XY,ish t(14;22)(p11.2;q13.33)(D22S1726+,ARSA+;D22S55+,D22S1726-,ARSA-). Interestingly a 22qter partial trisomy was identified on a proband's brother affected with Asperger's syndrome, including a very precious language, rich vocabulary and pedantic speaking style. So, one or more genes localised in this critical terminal 22q region might be implicated in language development through gene dosage effect.

P0707. Peculiar facies and mental retardation in mother and daughter: a possible new syndrome?

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The Authors describe a couple mother-daughter (35 and 11 yrs old respectively), whose phenotype is characterized by a peculiar facies, minor anomalies of the limbs and mental retardation in a medium range. Both of them have broad nasal bridge, upslanting palpebral fissures, high cheekbones, bulbous nasal tip, anteverted nares, long philtrum, thin and inverted V-shaped upper lip and full lower lip: they appear to be of South East Asian origin rather than Caucasian (which they are, also because of their olive complexion). They have a rough voice; hands with short digits(<3rd centile), partial skin syndactyly and clinodactyly of the 5th; stocky feet, with a large big toe. The mother's final stature is154cm, quite below her genetic target. The daughter is growing in the 10th centile (Tanner B1,P1). Behavioural alterations have been noticed: attention deficit, hyperactivity, anxiety in both and depressive tendency in the mother. A brain MRI does not show anomalies in the daughter, while the mother's left cerebral hemisphere appears underdeveloped, with a "simplified" cortical design. EEGs are alterated with no correlated clinical signs. A good quality, 450 G bands karyotype appears normal in both our propositae; fragile X is negative; the research of subtelomeric rearrangements is in progress.

Using a computerized dysmorphology database, no condition had a combination of such peculiar facies, minor anomalies of the limbs and mental retardation. To the best of our knowledge it looks like we are in front of a recognizable and transmissible syndromic pattern, possibly unique.

P0708. Angelman syndrome: a genetic and clinical survey of 243 patients.

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Angelman syndrome (AS) is a complex neurodevelopmental disorder with a difficult clinical diagnosis and a heterogeneous genetic basis. More than 70% of patients carry a 15q microdeletion detectable by FISH. Up to 5% of patients have maternal UPD, identifiable by segregation analysis of chromosome 15 microsatellites. Up to 5-10% of cases are due to an abnormal imprinting process. M-PCR test reveals all genetic defects except mutations in the UBE3A/E6-AP gene, which are responsible for the remaining 20% of cases. We report on 243 patients with a clinical diagnosis of AS analyzed by one or more of the following techniques: FISH, M-PCR, chromosome 15 haplotyping, UBE3A mutation screening. In order to define the recurrence risk in IC patients, microdeletions were tested by Southern-blot analysis and sequencing of the minimal overlapping region of AS deletion. Fifty-five cases negative just

by FISH or M-PCR were excluded by further analyses because of insufficient diagnostic criteria at clinical re-evaluation. A laboratory test confirmed the diagnosis in 66 out of the remaining 188 patients, with 42 deletions, 18 (including 4 familial cases) mutations of UBE3A/E6-AP gene, 5 defects of the imprinting process and one maternal UPD. We think that the low percentage of positive cases is likely due to scarce diagnostic criteria leading to misdiagnosis: work on a check list of clinical signs which can predict AS-specific genetic lesions is in progress. We also performed comparison of the clinical manifestations of our confirmed AS patients across the different subgroups aiming at assessing a putative phenotype-genotype correlation.

P0709. sleep and its disturbances in Angelman syndrome

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Angelman syndrome (AS) is a severe neurogenetic disorder, characterised by severe mental retardation, ataxia, seizures, absence of speech and characteristic facial features. We have studied sleep behavior of 62 AS patients aged 2 to 20 years through sleep questionnaires filled by the parents. Bedtime was between 8 and 10.30 p.m. , awakenings between 6 and 9 am, these results were similar to control group (30 healthy children aged 2 to 20 years), 53% of the children woke-up 1 to 3 times each night, and need parent's attendance to go back asleep. These sleep disturbances have a major impact on parents and family members, who report tiredness, depression, becoming sleep-deprived themselves. After the age of 3 years, children had no naps during the day , and do not objective fatigue. Adding anti-epileptic medication, 42% of the patients take different medications for sleep. Patients had maternal deletion 15q11-q13 (84%), paternal disomy (10%), imprinting defects (1%) or mutations of the *UBE3A* gene (4%). There was no phenotype-genotype correlation for sleep disturbances in AS. The mechanism of sleep disorders in AS is complex, insomnia may be related to abnormalities in brain development which may include abnormal function of the circadian system and seizures play a part in night awakenings. Psychological impact of lack of language could find its expression during the night, pointing to anxiety. Therapeutic management of sleep disorder in AS remains a necessity for the physician and parents.

P0710. Cellular mosaicism of maternal 15q11 imprinting in an atypical Angelman syndrome without obesity

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Atypical forms of Angelman syndrome with obesity, muscular hypotonia and ability to speak have been reported in patients with methylation pattern of the 15q11 region which supports the hypothesis of cellular mosaicism for a maternal imprinting (Eur J Hum Genet 1999, 7, 638) . We herewith report a new case with the same pattern of the 15q11 methylation, which extends further the clinical pattern of such anomalies.
The girl was born from healthy parents aged 37 years at conception and she is the second child. Pregnancy was uneventful. Length and birthweigth were normal. She walked unaided at 18 months. First words were said at 12 months. Language did not develop thereafter. At 3 years 6 months her weight and height were at the mean for the age. Her head circumference was at -1 sds for age. She exhibited mainly hyperactivity and was joyful. Cytogenetic analysis was normal. Because of the behavior characteristics the methylation pattern of the 15q11 SNRPN was evaluated. The maternal band was found fainted on three different samples (SNRPN exon alpha, Southern blot, XbaI and NotI double digestion). Methylation-specific PCR at the SRPN locus confirmed a faint maternal band. Quantitative Southern blot analysis of the critical IC region (AS-SRO) showed a normal dosage. Therefore IC deletion is unlikely. Sporadic imprinting defect and cellular mosaicism are likely explanations for such a pattern.
The present observation without obesity further extends the clinical delineation of this anomaly.
Ackownledgement: We thank the help of B Horsthemke, Institut fur Humangenetik, Universitatklinikum, Essen

P0711. A 17p11.2 deletion associated with a mild Smith-Magenis phenotype and GH deficiency.

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A 7-years old girl was referred for chromosome analysis because of recurrent infections and microcephaly. High resolution banding revealed an interstitial deletion in 17p11.2. The deletion was confirmed by FISH with the SMS probe (Oncor).
The girl presents with few facial dysmorphic features: anteverted ears, full upper lip with a "tented" appearance and mild micrognathia. Overall facial "gestalt" does not resemble the Smith-Magenis Syndrome (SMS). Thyroid function is in the normal range, while GH secretion was reduced upon stimulation. Her height, at 3rd percentile until 5.5 years, has improved to the 10th percentile after starting replacement therapy. She is microcephalic (below the 3rd percentile) and has short hands. Immunity function is normal apart from a IgA value at the lower limit of normal range. The girl had occasional absence-like episodes and repeated EEGs showed non-specific alterations. Psychomotor development evaluation revealed a borderline I.Q. She was diagnosed with ADHD (Attention Deficit Hyperactivity Disorder); she has temper tantrums, occasional stereotypic behaviours and mild sleep disturbances, while she never attempted to self-injurious behaviours, typical of SMS. FISH, aimed at defining deletion extent and boundaries, was carried out with YACs 795c9 and 828b9 mapping in tel cen order within the SMS deletion interval. Both signals were given by y795c9, while a strongly diminished signal was detected on one17p by means of y828b9, pointing out that the deletion is more centromerical than the typical SMS one. Further FISH experiments are in progress in order to address the genotype-phenotype correlation on such peculiar 17p microdeletion case.

P0712. Results of subtelomeric screening in 52 families with unspecific mental retardation.

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Recently, it has been shown that subtelomeric chromosome rearrangements may be a common cause of idiopathic mental retardation (MR). Such aberrations were found in ~7 % of patients with severe or moderate MR and dysmorphic features. However, further studies are needed to elucidate their prevalence and to establish more cost-effective selection criteria for subtelomeric test. We report results of screening studies in 52 families with idiopathic MR. Apart from MR, the main inclusion criteria were clinical features suggestive of a chromosomal aberration. Fluorescence in situ hybridisation with Chromoprobe Multiprobe T System (Cytocell) was used. In five cases subtle rearrangements were found. Verification of the karyotype at a higher resolution level has revealed that two rearrangements were half cryptic and in two cases both products of identified translocation could be seen. In one of them it was not a subtelomeric rearrangement but it could not be seen in the routine karyotype because of similar banding pattern and the size of involved regions. The prevalence of subtelomeric abnormalities was 4/52 (7,7%). All identified aberrations were familial in the origin and no abnormalities were shown in patients with mild MR. Our results confirm previous findings, indicating the important role of subtelomeric rearrangements in the aetiology of MR. They also emphasise the preferential occurrence of such abnormalities in patients with severe MR and positive family history for intellectual disability. Moreover, they also show that higher banding resolution should be used in the routine karyotyping in this group of patients.

P0713. 14q32.2 subtelomeric deletion in a child with severe congenital anomalies and a cryptic t(Y;14)(p11.3;q32.2).

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We describe a newborn with a de novo subtelomeric deletion of the terminal portion of the long arm of chromosome 14 due to a t(Y;14)(p12.3;q32.2).
The child was born at 36 weeks of gestation from healthy non-consanguineous parents. Fetal ultrasound examination at 28 weeks showed intrauterine growth retardation and anhydramnios. At birth the child demonstrated: weight 1160 g (<3th centile), length 40 cm (<3th centile), head circumference 28,5 cm (<10th centile), short nose with broad nasal root, low set dysmorphic ears, hands fingers anomalies, male external genitalia, undescendent left testis, imperforate anus and interventricular defect; umbilical cord was mono-arterial. The child died on day 19th .
Cytogenetic analysis using GTG tecnique revealed a 45,X karyotype. Because of the presence of external male genitalia, a FISH analysis with a SRY specific BAC probe (PHU 14) was performed, showing the presence of fluorescent signal in 14q telomeric region .
Further FISH analysis carried out with YAC and BAC probes specific for 14q32.3-qter region revealed a subtelomeric deletion with a breakpoint beyond 116 cM.
Father's karyotype with GTG and FISH analysis with PHU 14 probe was normal.
At least ten patients with a 14q terminal deletion have been reported with some common clinical features: an exact characterization of the deleted segment in our case can contribute to the delineation of a distinct 14qter deletion syndrome.

P0714. Subtelomeric Chromosome Abnormalities in Patients with Developmental Disorder

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Subtle chromosomal abnormalities have been reported to occur in 7.4% of patients with moderate to severe mental retardation as identified with a multiprobe telomere FISH protocol (Knight et al. 1999). We have studied 69 patients with mental retardation or developmental delay, and non-specific dysmorphic features, who had a normal 550 band G-banded karyotype. FISH testing was performed using the Cytocell Multiprobe-T system. Clinically significant abnormalities were found in six patients (8.7%, see Table). Three cases were familial. In addition we detected a common familial polymorphism del(2)(q37.3) in four patients (5.8%).

Patient	Karyotype	Parents
1	46,XX,der(2)t(2;10)(q37.1;q26.3)	maternal balanced translocation
2	46,XY,der(9)t(9;19)(q34;p13.3)	paternal balanced translocation
3	46,XX,der(18)t(2;18)(p25.3;q23)	normal karyotype
4	46,XX,del(1)(p36)	normal karyotype
5	46,X,der(X)ins(X;9)(q22;p24.2p24.3)	maternal 46,X,der(X)ins(X;9)(q22;p24.2p24.3)
6	46,XY,del(20)(q13.3)	normal karyotype

Detailed clinical findings of the patients and their relatives will be presented.
Subtelomeric FISH analysis proved to be a useful method in the detection of cryptic subtelomeric changes and provides a means for genetic counselling and prenatal diagnostics.

P0715. A novel method for the detection of subtelomeric rearrangements: Subtelomere COBRA FISH

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Cryptic subtelomeric chromosome rearrangements have recently been implied in the aetiology of mental retardation. Systematic FISH screening techniques to detect such rearrangements using subtelomeric probes have been introduced. To facilitate a more efficient analysis, multi-colour hybridisation techniques for subtelomeric probes are being developed. COBRA (COmbined Binary RAtio labelling) is a recent multi-colour FISH labelling technique which combines ratio and combinatorial labelling to attain especially high multiplicities [Ref. 1, 2].
With the novel "Subtelomere COBRA FISH", the specific detection of all necessary 41 BAC and PAC FISH probes (second generation probe panel, Ref. 3) is possible in only two hybridisations. By strict probe selection and characterisation and by using the ULS / Universal Linkage System ® labelling technique, high specificity and hybridisation efficiencies could be reached. This allows the unequivocal analysis of 21 respectively 20 probes per hybridisation with only two to three metaphases each. In contrast to other subtelomeric multi-colour techniques, the high multiplicities of Subtelomere COBRA FISH make it possible to differentiate e. g. long arm and short arm subtelomeric regions of a given chromosome thus permitting the diagnosis of cryptic pericentric inversions in addition to translocations and deletions.
The technique, its validation and first results will be presented.
References: [1] Eur J Hum Genet (1999) 7: 2-11, [2] Genome Res. (2000) 10:861-865, [3] Am J Hum Genet (2000)67:320-332
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P0716. Studies Of Telomeric Fish Screening And High Resolution Cgh In Populations With Recurrent Miscarriages Or Mental Retardation

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Aberrations of chromosomal telomeres are a frequent cause of mental retardation, particularly if they are associated with dysmorphism. It has been postulated that they could also account for recurrent miscarriages.
OBJECTIVES: The goal of this study is to use and compare both Fluorescent In Situ Hybridisation (FISH) and high resolution Comparative Genome Hybridisation (CGH) in 2 populations: children with mental retardation, and couples having had at least 3 unexplained miscarriages.
PATIENTS : 200 children with idiopathic mental retardation and 57 couples (114 patients) were studied after informed consent according to the Helsinki convention. Of 200 patients 29 were excluded because they had abnormal karyotype, non-telomeric microdeletion or a sib in the study.
METHODS: FISH was performed using the Cytocell or Adjenix probes. CGH was performed using the Adjenix Nick Translation kit and the Cytovision Image Analyser.
RESULTS: In the mental retardation group 13/171 anomalies were found or 7.6% using FISH. Anomalies were often familial and 5 involved chromosome 22q. CGH was performed in 50 patients: 39 picked at random and 11 to validate the method. One familial microdeletion was found involving chromosome 17. 7 couples had an abnormal Karyotype but no FISH anomalies were found in the miscarriage group.
CONCLUSIONS : Telomeric FISH screening is useful for diagnostic and prognostic purposes in children with mental retardation and normal karyotype resulting in 7.6% anomalies. It does not seem useful for couples with recurrent miscarriages.The value of CGH will be discussed.

P0717. FISH characterization of 16p11.2-p12.2 tandem duplication in a dysmorphic patient with severe mental retardation and autistic behaviuor.

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Chromosome 16p partial trisomy is a rare anomaly, described in

the literature in less than 30 patients. We report on the genotype/phenotype correlations drawn on a 23 years old dysmorphic male affected by mental retardation and autistic behaviour, who was found to have a 46,XY,add(16)(p13) karyotype, redefined as 46,XY,dup(16)(p12.1p12.2). The detailed clinical evaluation showed the presence of hypertelorism, broad nasal bridge and tip, short phyltrum, macrostomia, thick lips, short stature, joint laxity. FISH analysis by using a WCP and a PCP chromosome 16 probes confirmed duplication of 16p11.2-12 region. FISH studies with YAC probes belonging to WC16.2 contig and BAC probes mapping in the region between 16p12.3 and 16p11.2 allowed to restrict the duplication to the 16p12.1-12.2 region; the inclusion of 16p11.2 band in the duplicated fragment is still under test. The comparison between clinical features of 16p trisomic patients in the literature and those of our patient carrying a precisely characterized chromosomal abnormality can improve the karyotype/phenotype correlations for 16p imbalances. The absence of microcephaly, heart and genital abnormalities in our patient and in the other one with a similar16p anomaly, previously reported, suggests that distal 16p is involved in the pathogenesis of these anomalies, while the presence of severe mental retardation with autistic behaviour, may be attributed to more centromeric 16p sequences.

P0718. Clinical, cytogenetic and molecular analyses of partial 21q monosomy in a girl with mental retardation, marfanoid habitus and minor dysmorphic features

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Phenotypic and molecular analysis of patients with partial monosomy 21 resulting from translocations, ring chromosomes or pure partial monosomy 21 allows to define which regions of chromosome 21 contribute to the generation of specific aspects of the 21q- syndrome. Here we report on the clinical, cytogenetic and molecular characterization of a `pure´ de novo partial monosomy 21 with a deletion of 21q22.2-qter. FISH mapping of cytogenetically and genetically anchored YAC and BAC clones resulted in the identification of a breakpoint spanning BAC clone. Our FISH results clearly showed that the deletion breakpoint is located distal to ETS2 gene, in the proximal part of the region 21q22.2. Furthermore molecular studies using polymorphic markers supported these findings and showed that the derivative chromosome 21 was of paternal origin. The patient who presented with mild mental retardation, marfanoid habitus and minor dysmorphic features, is lacking most of the typical features seen in the 21q- phenotype and thus is quite unique. Our findings support the suggestion that the loss of the region at 21q22.2-qter is critical for only some minor aspects of the 21q- syndrome. Genotype-phenotype correlations of our case and other reported cases will be discussed.

P0719. Unbalanced subtelomeric translocation 11q;16q in a mildly retarded boy with severe speech delay and minor dysmorphic signs

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We report on a mildly retarded boy, whom we investigated at the ages of 2 and 4 1/2 years.
He is the first child of a healthy non-consanguineous couple. The mother is currently pregnant.
After an uneventful pregnancy and delivery by caesarean section, measurements were normal (3130g , 47,5cm, 34cm). Neonatally, the boy developed meningitis. His craniofacial dysmorphism included a high forehead, slightly depressed, broad nasal bridge and tip with anteverted nares, thick lips, small mandible and high arched palate. He had pes equinovarus, mild muscular hypotonia and right kryptorchidism.
His motor development supported by physiotherapy was normal (crawling with 6 months, sitting with 8 months, walking with 13 months). He suffered from repeated otitis media and his hearing was severely impaired. His active and passive verbal development is severely delayed.
Screening for metabolic diseases and repeated chromosomal analyses were normal as were parental chromosomal analyses. Brain MRI showed periventricular leucodystrophy.
A screening for cryptic subtelomeric chromosome aberrations (Vysis ToTelVysion) detected a cryptic unbalanced translocation 11qter / 16qter causing partial monosomy 11q25 and partial trisomy 16q24. Parental analyses demonstrated a maternal balanced translocation t(11;16)(q25;q24)(VIJyRM2072-,16QTel013+;VIJyRM2072+,16QTel013-). Both findings were confirmed by the novel Subtelomere-COBRA multi-colour FISH method. Further family studies including the analysis of the ongoing pregnancy will be presented.
Given the proband's relatively mild phenotype, the results emphasise the importance of subtelomere studies in mildly retarded patients.
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P0720. Molecular cytogenetic characterization of two cases with cryptic rearrangements of the 6q subtelomeric region.

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We report two cases with cryptic rearrangements of the 6q subtelomeric region characterized by using FISH techniques.
Case n. 1 is a 28 years old male with: severe mental retardation, hypoplasic malar regions, cupid bowed upper lip, everted lower lip, micrognatia, large, anteverted and low set ears with prominent anti-helix, surgically corrected nasal dysmorphisms and slight bilateral clinodactyly. Standard karyotype and molecular test for FMR1 gene appeared to be normal.
FISH analysis for subtelomeric regions using the Cytocell Multiprobe T System showed the 6q deletion that was revealed to be de novo. A further characterization of the breakpoint was carried out with 6q27 region YAC and BAC probes.
Case n. 2 is a 9 years and 9/12 male child showing severe psycomotor retardation, patent ductus arteriosus, vescico-ureteral reflux, frontal bossing, short palpebral fessures, lateral displacement of inner canthi, flat nasal bridge, big and low set years with prominent anti-helix, triangular mouth, long upper lip, micrognatia, barrel thorax, scoliosis, wide spaced and low set nipples, bilateral hip dislocation, clubfoot, campto-clinodactyly of toes. Standard karyotype was normal while the FISH analysis by using the Cytocell Multiprobe T System evidenced a 2q deletion and a 6q duplication.
These two cases stress the necessity of using specific subtelomeric FISH probes to detect cryptic rearrangements in patients with syndromic mental retardation.
The precise characterization of the involved regions by using molecular techniques may be useful in order to clinically define new microdeletion/microduplication syndromes.

P0721. FISH analysis of replication and transcription of chromosome X loci in Rett syndrome

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Differential replication staining using BrdU + Hoechst 33258 technique has been carried out on a series of 60 girls with Rett syndrome (RTT). The results indicated that regions Xq23 and Xq28 of inactive chromosome X could contain early replicating and, therefore, transcriptionally active loci at RTT. Interphase fluorescence in situ hybridisation (FISH) studies of replication timing, using chromosome X specific genomic DNA probes, was applied to determine the loci with altered replication and transcription at RTT. 14 randomly selected PAC clones for Xp, Xcen and Xq were used. Two clones from Xq28 (anonymous clone PAC 24.23.0 and PAC 671D9, containing MeCP2 locus), probably, escape inactivation in late-replicating chromosome X in RTT patients. Therefore, region Xq28 could contain the genes escaping X- inactivation and expressing from the human active and inactive chromosomes X. These results support the hypothesis proposing the disturbances in dosage compensation effect due to

aberrant activation of genes in inactive chromosome X at RTT genes (di-allelic expression instead of mono-allelic). Our results indicate that MecP2 itself could escape X-inactivation and reduce the pathogenic effect of mutated allele at RTT. Supported by Copernicus2 and INTAS grants.

P0722. Molecular-cytogenetic studies of Rett syndrome (RTT) in Russia: the investigation of 4 boys and 81 girls

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Rett syndrome (RTT) is a severe neurodevelopmental disorder with the incidence of 2.5% in mentally retarded girls in Russia. We have performed cytogenetic studies of 85 patients (81 girls and 4 boys) with clinical picture of RTT. Molecular analysis in 30 randomly selected RTT patients revealed that 84% of them have mutations in MeCP2 gene. Among 85 patients: 81 girls with clinical picture of RTT were with normal female karyotype (46,XX); two boy was with normal male karyotype in cells of blood (46,XY) and two boys were with mosaic forms of Kleinfelter's syndrome (47,XXY/46,XY) in blood and muscle cells. 24 mothers and parents of RTT girls were with normal karyotype, three mothers - with mosaic form of Turner syndrome (45,X/46,XX), mosaic form of trisomy X syndrome (46,XX/47,XXX) and one - mosaic karyotype -47,XX,+mar/48,XXX,+mar. We analysed chromosome X in lymphocytes of 81 affected girls with clinical picture of RTT using BrdU + Gimsa staining technique. Specific type of inactive chromosome X (so-called type "C") with unusual staining of chromatin in long arm of the chromosome X was found in 76 (93%) girls with RTT. This technique was positively used for presymptomatic diagnosis of RTT in five girls in affected families. We believe that the phenomenon of altered chromatin conformation in inactive chromosome X could be used as laboratory test for preclinical diagnosis of the RTT. Supported by Copernicus2 and INTAS grants.

P0723. Crytic translocation resulting in Angelman syndrome: implication for genetic counselling

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Angelman syndrome is a well-characterised neurobehavioral disorder, associated with different abnormalities: large de novo maternal deletions of 15q11q13, paternal uniparental disomy of chromosome 15, mutations in the UBE3A gene and imprinting defect. Most Angelman syndrome cases result from de novo deletion related to the presence of repeat elements, duplicons, flanking 15q11q13 region.
However, few reported cases demonstrate that deletions may be the result of cryptic structural chromosomal abnormality which involve the 15q11q13 region. This notion led us to systematically control the maternal chromosome 15 structure with molecular cytogenetic method. This strategy allowed us to identify a patient with 15q11q12 deletion resulting from malsegregation of cryptic maternal reciprocal translocation between chromosome 15 and 22: 46, XX, t(15;22)(q12;q11.2).
This observation illustrates the necessity to currently used molecular cytogenetic method to detect such rearrangement taking account their high recurrence risk.

P0724. Methylation-sensitive multiplex FRAXA-FRAXE PCR assay is a powerful non-invasive neonatal screening method capable of detecting genetic abnormalities in 1: 500 newborn boys.

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Fragile X syndrome (FRAXA mental retardation) is the most frequent form of inherited mental retardation. Discovery of molecular defect causing this disease in 1991 allowed development of precise laboratory diagnostics methods. Nevertheless, for many years these methods remained expensive, time- and labor-consuming. This hampered the development of fragile X screening programs, the necessity of which is widely accepted.
We present a molecular test for fragile X syndrome as well as for FRAXE mental retardation based on FMR1/FMR2 promoter methylation detection via methylation-sensitive PCR. It may be used not only for fragile X diagnostics, but also as a non-invasive screening test on umbilical cord blood to identify fragile X (FRAXA and FRAXE) patients and those carrying extra X chromosome(s) among newborn boys (47,XXY; 48,XXXY; 49,XXXXY; 46,XX male karyotypes and mosaic variants). It may become a powerful tool for detecting fragile X carriers alternative to overall screening of young women, for the latter is associated with serious technical, ethical and financial problems. The methylation-sensitive test proposed here is one of the most efficient and cost-effective screening methods, for it allows detection of genetic abnormalities with a total incidence of approximately 1:500 and is at least tenfold less expensive then conventional molecular detection of fragile X or extra X chromosomes in males. Introduction of such a test would allow early adequate therapeutic and psychological activities towards boys with frequent forms of X-linked genetic abnormalities and, in combination with cascade screening in families, reduce considerably the incidence of the fragile X syndrome.

P0725. X inactivation and fragile X methylation in human placentas

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In female somatic cells X inactivation is associated with differential CpG methylation on the X chromosomes, e.g., the LINE1 element of the DXS255 minisatellite is extensively methylated only on the active X whereas CpGs in the androgen receptor and the FMR1 gene are methylated only on the inactive X chromosome. Full expansion of the FMR1 CGG repeat in male and female fragile X patients is usually associated with promotor hypermethylation. In chorionic villi of female placentas, X inactivation methylation differs significantly from somatic cells. Also, differences between X inactivation and full mutation methylation of FMR1 have been reported. We have evaluated the methylation status of FMR1 and other X linked loci in chorionic villi from first trimester and full term female placentas of normal and fragile X pre/full mutation individuals. In contrast to somatic tissues, X inactivation methylation was absent from DXS255 and from the FMR1 promotor at any stage of development while methylation was present on other loci. In contrast to X inactivation methylation, fragile X full mutation hypermethylation has been detected in a 13 week old male fetus but was frequently not seen in the first trimenon. Human chorionic villi are hypomethylated. Differential methylation and inactivation of X-linked genes seems to depend on tissue and locus specific methylase interaction as proposed for fragile X hypermethylation.

P0726. Prenatal diagnosis of X-linked Opitz G/BBB syndrome

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Opitz G/BBB syndrome (OS) is an inherited disorder characterized by midline defects and psychosomatic retardation. OS is genetically heterogeneous with a X-linked and an autosomal form known (respectively Xp22 and 22q11.2). We describe here the first (to our knowledge) reported case of prenatal diagnosis of X-linked OS.
A 9 year-old Greek boy presented with typical signs of OS, including mental/growth retardation, hypertelorism, strabismus, epicanthus,narrow palpebral fissures,flattened nasal bridge, flat filtrum, micrognathia, cranial assymetry and brachycephaly, prominent forehead, low set ears, oesophageal stenosis, heart defects, corpus callosum hypoplasia, seizures, hypotonia, and hypospadias.There was no family history with similar cases.The

boys' parents were apparently normal, unrelated, and had no other child.Another pregnancy was terminated at 6 months, because US revealed that the male fetus presented with laryngotracheal cleft and clubfoot.The boy had a normal karyotype, and FISH analysis of the DiGeorge region of 22q11.2, revealed no abnormalities found in autosomaly inherited OS cases.We therefore searched for a mutation in the MID1 gene, which is responsible for X-linked OS. SSCP analysis of the whole gene revealed a child's DNA segment of 345 bp with an abnormal electrophoretic pattern compared to normal controls and his father.The boy's mother was heterozygous for the abnormal segment.
The parents asked for prenatal testing for OS. DNA from CVS was obtained at 12th week of pregnancy and screened for the mutant MID1, in addition to two chromosome Y genes.The fetus was male and had the OS mutation, so after genetic counseling, the parents decided to terminate the pregnancy.

P0727. A rheostat model for a rapid and reversible form of imprinting-dependent evolution

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The evolutionary advantages of genomic imprinting are puzzling. We propose that genomic imprinting evolved as a mechanism that maximizes the inter-individual variability in the rates of gene expression for dosage-sensitive loci that can alter the phenotype over a wide continuum with minimal unrelated deleterious effects, and we refer to this as a rheostat model. We hypothesize 1) that genomic imprinting provides a haploid selective advantage (HSA) - not an original proposal; 2) that many imprinted genes have evolved genetic and epigenetic mechanisms that facilitate quantitative hypervariability (QH) of gene expression; 3) that the combination of haploid selective advantage and quantitative hypervariability makes possible a rapid and reversible form of imprinting-dependent evolution (IDE) that can mediate changes in growth, behavior, and perhaps other traits; 4) that this enhanced adaptability to a changing environment provides selective advantage as an assisted form of evolution; and 5) that these mechanisms have provided at least one of the driving forces for the evolution of genomic imprinting in mammals. The rheostat model suggests some nontraditional genetics including both genetic and epigenetic variants contributing to an integrated mechanism of mixed Mendelian and non-Mendelian inheritance, the possibility that the majority of variants are not intrinsically deleterious but are each potentially advantageous depending on the environment, a reversible form of assisted evolution, and the ability to protect a silent allele from selection for many generations but reactivate and expand it in the population quickly.

P0728. Characterization of a novel brain specific transcript as candidate for imprinting

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We have identified a novel human gene on chromosome 20q13, a region known to be syntenic to distal mouse chromosome 2 containing imprinted genes. The human transcript is strongly expected to be brain specific as Northern analysis of 8 tissues revealed expression of a 3.2 kb and a 3.0 kb transcript in brain only. The corresponding cDNA (AJ311122) contains a 1680 bp ORF distributed on 13 exons spanning a genomic region of approximately 250 kb. The homologous mouse cDNA (AK005136) contains a 1680 bp ORF either, and multiple tissue Northern analysis revealed 3 major transcripts of 3.3 kb, 2.9 kb and 2.5 kb visible in brain only emphasizing the human expression pattern. During detailed expression analysis using 8 different brain specific tissues a complex pattern has been detected concerning the quantity of every single transcript depending on analyzed tissue. A developmental specific expression pattern has been found during embryogenesis displaying weak signals from day 10 pc and strong signals from day 15 pc onwards to adult mice suggesting a function from late development. Database analysis using translated human and mouse gene products revealed homology to several hypothetical proteins of yet unknown function sharing a striking homology of approximately 120 aa at the C-terminal end suggesting the existence of a shared domain, evolutionary conserved down to D. melanogaster and C. elegans.
This work was supported by DHGP.

P0729. Prevalence of fragile-X female carrier in Taiwan is lower than expected

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Fragile X syndrome (FXS) is not treatable today, but can be prevented by prenatal genetic examination. Identifying female mutant carrier before or at early pregnancy through a wide screening program is considered a practical approach. However, the carrier prevalence in a population and cost of screening test should be carefully evaluated prior to implementation of such a program. To ascertain the prevalence of FXS in Taiwan, we screened a total of 1002 pregnant women using a high-resolution Southern blot test to examine the pooled DNA, and a simple non-radioactive PCR test to identify heterozygous women (*Tzeng et al; Diagn Mol Pathol 2001;10:34-41*). From these women, we did not find any carrier of premutation and/or full mutation. There were 22 women with an allele exhibiting CGG-repeat between 40 and 52, including two with 48 and three with 52. Approximate one third of the women could be rapidly excluded from being a carrier with the simple PCR test by proving both their alleles were different and within normal CGG-repeat range. This is the first study reporting the female fragile-X carrier rate in Asia population, with a result indicating that the carrier rate in Taiwan is lower than that reported from Israel, Finland, Canada, and the United States, ranging between 1:113 and 1:320. Therefore, we do not recommend such a program to screen general population in Taiwan. Whether it is worthy for women with family history of mental retardation with undermined cause needs to be further investigated.

P0730. 5 years of molecular diagnosis of Fragile X syndrome (1997-2001): a collaborative study of 22 laboratories in France.

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The fragile X syndrome is the most common cause of inherited mental retardation. A preliminary study of the efficiency of the screening in mentally retarded probands with no previous familial diagnosis was done in Strasbourg with a comparison between data from 1991-1994 and 1997-2000 (1058 and 2771 families analyzed respectively). This comparison showed a quite stable efficiency of the diagnosis since 1994 (between 1.5 to 3.2% of positive cases), and some improvements in its precocity and exhaustiveness with a decrease of the age at diagnosis of the proband (average :16 to 12 years) and an increase of the percentage of families detected with only a sporadic case (35 to 65%) or with a female proband (7.7 to 15%).
This study was enlarged to 23 laboratories and we will present the results covering 5 years of screening. The preliminary data from 18 laboratories are in agreement with the above results. 15517 families

were studied, and allowed the identification of 351 FraX probands. The efficiency is 2.26%. The average age of FraX probands detected was 11. The detection concerned a sporadic case in 56% of the families, and a female in 12%.
225 of the 351 families were analysed further in each laboratory, leading to the testing of 463 females at risk, among whom 338 carriers of premutations or full mutations were diagnosed, and to 52 prenatal diagnoses.
The data from the 23 laboratories will be presented, with issues concerning premature ovarian failure and premutation discovered in a mentally retarded proband.

P0732. Five years experience with DIG labeled probes on Southern blots applied in Fragile X diagnostics

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The DIG labeling and detection system is a simple system to detect single copy genes on Southern blots. DIG labeled probes are at least as sensitive as 32 P labeled probes, they are very stable and faster and safer to work with. In our hands, incorporation of DIG-dUTP by PCR is the most preferred labeling method, which generates highly specific probes. The hybridized probe can be detected after hybridization with an alkaline-phosphatase antibody and CDP-star as a substrate. We have been using the DIG technique in our diagnostic tests for fragile X syndrome. Since this is a triplet expansion syndrome with a wide range of fragment lengths, it is a critical application for Southern blotting. The DIG labeling method proved to be a reliable single test that discriminates between normal alleles, premutations, full mutations and mosaics. Postnatal screening involved > 2000 samples and we identified 30 patients and 31 carriers. We did 26 prenatal diagnoses and found 8 affected and 3 carrier fetuses.

P0733. Analysis of FMR 1 methylation in Fragile X Syndrome in Iranian Population

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Fragile X syndrome is the most common cause of inherited mental retardation. This syndrome is knows to be the result of a dynamic trinucleotid mutation at the 5' UTR (Untranslated region) of the FMR1 (Fragile X Mental Retardation) gene.
We use PCR as a pre-screen and only to proceed to southern blot on those sample which fail to amplify (males) or show a single normal alleles (females). The remaining samples were subjected to southern blot analysis that often combined with methylation analysis by restriction enzyme digestion with a methylation-sensitive enzyme. Determining methylation status EcoR I is combined with a methylation sensitive enzyme Nru I was used.
In both procedures PCR and Southern Blot analysis non- radioactive protocol were used PCR product were detected by silver staining and digoxignin was used in Southern Blot.
Total of 275 individuals from 200 families with at least one mentally retarded child were examined 110 case had a full mutation, 17 with a permutation and 148 were normal. In prenatal diagnosis that was performed for 8 fetuses from these families 2 normal males, 1 normal females 3 fullmutation males and 2 fullmutation females were detected.

P0734. CGG-repeat expansion and metilation status of the promotor region of FMR1 gene analysis in the Fragile-X sindrome patients from Ukraine

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FRAXA is folate sensitive fragile site, which associated with X-linked mental retardation. FMR1 gene, whose 1-st exon includes the FRAXA site on Xq27.3, accounts for nearly 20% of all X-linked forms of mental retardation. The fragility in this site is due to expansion of CGG-reports, which associated with hypermethylated CpG islands. For this study we selected the group of patients with Fragile-X syndrome phenotype from Ukraine. We detected CGG-expansion in FMR1 gene by Southern blot analysis using pX6 probe and/or direct PCR analysis in 14 causes from 79. We performed the methylation analysis for group of 8 patients with detected CGG-expansion or negative PCR analysis (CGG-expansion or deletion). Hin6.1 is a methylation-sensitive enzyme that cuts only unmethylated recognition sites. The PCR of Hin6.1-restricted DNA by use of primers flanking the FRAXA CpG islands amplifies only the non-restricted (inactivated) copies of CpG islands. We detected hypermethylation in samples with CGG-expansion (5/8) as well as in samples with negative PCR (3/8). This test is very simple, inexpensive and effective method. At our mind, this test would be useful for performing diagnosis, postnatal and prenatal diagnostics as a control of Southern blot analysis and direct PCR analysis and for screening patients with mental retardation and newborn boys.

P0735. Mutation spectrum in Rett syndrome in Denmark

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At present 88 patients have been registered in Denmark with the diagnosis Rett syndrome, 87 female patients and one boy. They were born in the period 1923 - 1999. Seven have died at ages between 11 and 65 years. Seventy-four have been screened for mutations in *MECP2* by sequencing the exons 2-4. In 62 patients a mutation was identified (84 %), 48 single base substitutions, 12 small deletions and two small additions. The single base substitutions were 23 nonsense mutations: Y141X(2), R168X(5), R255X(7), R270X(3) and R294X(6) and 25 missense mutations: R106W(4), R133C(2), S134C(1), T158M(11), P302L(1), R306C(5) and R309W(1).
R309W has not earlier been reported. The patient has a very mild variant form of Rett syndrome with only a few of the typical traits. There is a slight tendency towards a milder phenotype in the cases with a missense or a late truncating mutation. But it applies to all the recurrent mutations, R133C excepted, that the phenotype varies from mild to severe.
The oldest of the seven patients with the R255X mutation is 27 years old and still able to walk independently, while the youngest is 2 years old and probably will never be able to walk. The oldest of the 11 patients with the T158M mutation is 78 years old, she walked with support until she was 41 years old, while three of these patients, 2-9 years of age, have never walked independently.
For details about the boy, see abstract by Ravn et al.

P0736. Low frequency of MECP2 mutations in mental retardation of unknown origin: implications for routine DNA diagnostics

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Mutations in the methyl-CpG binding protein 2 (MECP2) gene are not always lethal in males. MECP2 mutations have been found in males with severe mental retardation with or without progressive encephalopathy, males with an Angelman-like phenotype, and males with mild nonspecific mental retardation. It was suggested that the frequency of mutations in MECP2 in mentally retarded males equals the frequency of the CGG expansion in the FMR1 gene. In order to determine if MECP2 screening should be implemented in a routine diagnostic setting for patients with nonspecific mental retardation, we tested a cohort of 500 male and 100 female mentally retarded patients who were negative for the expansions across the FMR1 CGG repeat. Furthermore, 70 mentally retarded patients with a clinical diagnosis of Angelman syndrome, but without a molecular abnormality on 15q, were included in this study. In each of these three patient groups only one causative mutation could be identified. Several amino acid changes appeared to be polymorphisms after testing unaffected male family members. Because one of these patients showed a Prader-Willi like phenotype, we performed MECP2 mutation analysis in 100 patients with a clinical but no molecular diagnosis of Prader-Willi syndrome. Until now no mutations have been found. We conclude that the incidence of MECP2 mutations in patients with mental retardation of unknown origin is low and we do not favour implementation of this gene in routine DNA diagnostics. Results of the mutation analyses and clinical findings in the patients with a MECP2 mutation will be presented.

P0737. Large Deletions of entire Exons of MECP2 gene, may represent the genetic defect in some RTT patients with no mutations found in the coding region by using DNA sequencing analysis

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Rett syndrome (RTT) is an X-linked progressive neurodevelopmental disorder. Affected females develop normally until 6-18 months of age, then gradually lose speech and purposeful hand use, and develop microcephaly, autism, ataxia, seizures, abnormal hyperventilation and stereotypic hand movements. Rett syndrome is caused by mutation in the MECP2 gene on chromosome Xq28 (Amir et al., 1999).
To date, mutations in the coding region of MECP2 account for RTT in 65-85% of the known cases.
We performed a long distance PCR coupled with long-read direct sequencing, to analize the entire MECP2 gene coding region in 101 unrelated RTT girls.
Mutations were identified in 79/101 patients, both with classic and non-classic phenotype.
Special attention was dedicated to the subjects with no mutation found in the coding region of MECP2 gene by sequencing analysis, using further approaches. In one family we found in the mother a polymorphic, common neutral variant C-A in the last codon, at the 3' of exon 3. The father does'nt shows the variant, and the RTT daughter has apparently only the maternal allele.
We developed a quantitative PCR based densitometric dosage assay, on a Long Readir LICOR -4200, to demonstrate the deletion of entire exons of MECP2 gene in this RTT patient.
Other approaches (FISH, Southern blotting and RT-PCR analyses) are now in progress to confirm this hypothesis.

P0738. Comprehensive mutation analysis of the MECP2 gene and the analysis of 54 Rett syndrome suspected patients

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Rett syndrome is an X-linked dominant neurodevelopmental disorder, affecting 1/10.000-15.000 girls, characterised by a period of early normal growth and development followed by regression with loss of speech and acquired motor skills, stereotypic hand movements and seizures. The disease-causing gene, mapped to Xq28, was identified as MECP2, encoding the methyl-CpG-binding protein 2.
To analyse this gene in patients suspected of Rett syndrome, we developed an efficient pre-screening method, based on denaturing gradient gelelectrophoresis (DGGE), followed by direct sequencing in case an aberrant band pattern is found. The DGGE system consists of 15 amplicons which all can be analysed under one single experimental condition.
Until now 54 patients have been analysed, whose clinical diagnosis varied from mental retardation to classic Rett syndrome. In 14 patients (26%) 10 different mutations have been identified, 3 nonsense mutations in 5 patients and 7 missense mutations in 9 patients. One nonsense mutation has not yet been reported, Q47X. In one patient two missense mutations have been found, both previously published as being the cause of Rett syndrome.
The overall mutation frequency is far below other published results. In view of our extensive experience in designing DGGE systems for mutation detection that result in the detection of virtually all possible mutations, this low frequency is most probably due to loose inclusion criteria. Clinical characteristics of the patients will be presented in comparison with the results of the mutation analysis.

P0739. Mutation analysis of MeCP2 gene in 36 patients with Rett syndrome of Slavic origin: Detection of two novel mutations and one new polymorphism

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Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder that almost exclusively affects girls. A prevalence is estimated to be 1:10, 000 to 1:15, 000 females. Patients with classic RS are characterized by a period of normal growth and development followed by regression with loss of speech and acquired motor skills. Neurologic abnormalities include spastic paraparesis, ataxia, intermittent hyperventilation, and epilepsy. Growth retardation, scoliosis, and autonomic dysfunction are common. RS is caused by mutations in X-linked MECP2 gene, encoding for methyl-CpG-binding protein 2. It plays an important role in the regulation of gene expression. The spectrum of mutations in MECP2 gene is known from numerous countries and ethnic groups and steps are being taken to determine the genotype/phenotype relationship in order to understand the disease process. Here we report mutation analysis of 36 patients with RS from the Czech and Slovak republics. Systematic sequencing of the entire coding sequence of MeCP2 gene revealed, in exon 4, thirteen different disease-causing mutations in 22 sporadic patients (61%). Two have not been previously published: a small deletion of 3 bp (1069delAGC), and a deletion of 172 bp along the insertion of 41 bp (1063del172bp+ins 41bp). Eleven patients had nonsense mutations (Y141X, R168X, S204X, R255X, R270X, R294X), eight carried missense mutations (R133C, K135E, T158M, R306C), and one had a frameshift mutation (1157del41bp). The novel polymorphism 587 C>G (T196S) was detected in a patient carrying the mutation 397 C>T (R133C). (Supported by Czech Granting Agency - GACR 301/01/P068 and LN00A079 from MSMT of Czech Republic)

P0740. Analysis of the MECP2 gene by Direct Sequencing in Hungarian Rett Syndrome Patients

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Rett syndrome is an X-linked neurodevelopmental disorder characterized by loss of acquired skills, stereotypical hand movements, microcephaly, trunk ataxia and hyperventilation. Epilepsy may also be present in some patients. Mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2) have been identified as cause of Rett syndrome. Based on these results we initialized mutation screening of MECP2 in Hungarian Rett syndrome patients. Patients of the Hungarian Rett Syndrome Association were involved in the present study. So far we have examined 19 patients, from various districts of Hungary, who were supposed to have Rett syndrome for MECP2 mutations. Initially a detailed clinical evaluation were performed including physical, neurological and orthopedical examinations, EEG and bone X-ray studies. Genetic studies included chromosomal analysis and FISH for Angelman syndrome. Routine metabolic screening and serum IEF for CDGS were also performed in every patient to exclude other underlying etiologies. Based on these scrutinized analysis, the clinical diagnosis of Rett syndrome could be supported in 15 patients out of the 19 cases. Mutations in MECP2 were detected in 8 cases. We found five already described mutations in six patients (R106W in two patients, P152R, R168X, R270X, R294X), a novel single base insertion in exon 3 (276insG) in a patient with a clinical history showing slow progression, and a large deletion in exon 4 in a patient with typical Rett syndrome. A new polymorhism (N126K) characteristic for the Hungarian population was also detected. This amino acid alteration was found in all examined patients so as in healthy controls.

P0741. A 10-year-old boy with classical Rett syndrome caused by a frameshift mutation the MECP2 gene.

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Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder, considered as exclusively affecting girls. Affected male fetuses were thought to be aborted spontaneously or to have a different phenotype. So far 17 males with a mutation in MECP2 have been reported so far. Five of these had the karyotype 47,XXY or were mosaic for the mutation. The remaining cases can be divided

into two groups; a severe type, who dies within the first years of life, all caused by an MECP2 mutation, which in females has be found to be associated with classical RS, and a mental retardation type with mutations, which if present in females are compatible with a normal or mildly retarded development. Here we present a 10-year old boy with clinical RS, with a normal karyotype, no signs of mosaicism and a truncating mutation 816dup7 in the MECP2-region encoding the transcription repression domain, TRD.
The resulting MeCP2 protein is predicted to contain a functional methyl-binding domain, but lacking most the TRD and the site facilitating MeCP2 binding to DNA.
While comparing the genotype-phenotype correlation in female with mutations in MECP2 is hampered by X chromosome inactivation, the phenotype of males hemizygous for the same mutations should shred light over the effect of these mutations on the phenotype. However, our patients harbour a typical RS mutation, but have survived beyond early childhood in contrast to the expectation and the experience for these mutations. The hypothesis that other genes are interfering with the clinical features of RS is possible.

P0742. Mutation or polymorphism in the MECP2 gene in mentally retarded boys : diagnostic implications

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Among the well characterized X-linked conditions causing mental retardation, mutations in the methyl-CpG binding protein 2 (MECP2) gene on Xq28 have been found in 70-80% of patients with Rett syndrome, a neurological disorder which, in addition to other symptoms, severely affects higher cognitive functions in females. Mutations in the MECP2 gene are involved in a broad spectrum of phenotypes from classical Rett syndrome to mild intellectual difficulties in feamles and neonatal encephalopathy in males . Recently, few MECP2 mutations were reported in males with non specific mental retardation suggesting that defects in MECP2 are responsible for about 2% of X-linked mental retardation.
To assess the frequency of MECP2 mutations, we screened, by DHPLC, the coding sequence and flanking regions of the MECP2 gene in a cohort of 262 mentally retarded males found negative for fragile-X syndrome.
First results show one recurrent intronic polymorphism (IVS3-19delA) and a nucleotide variant P376S. None of these sequence modifications were detected in 200 controls. Nevertheless, we already identified the nucleotide variant P376S, in a girl presenting an atypic Rett syndrome. A detailed familial study on three generations showed that the substitution P376S was also inherited by a healthy uncle, thus ruling out its involvement in the etiology of the disease. This finding clearly calls for a careful consideration of the pathogenicity of the MECP2 mutations identified in males before genetic counselling.

P0743. Drosophila as a model to study the physiological pathway in which FMRP (Fragile X Mental Retardation Protein) is involved

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The Fragile X mental retardation syndrome results from the absence of the protein (FMRP) encoded by the FMR1 gene. FMRP is an RNA binding protein, that has two close homologs, FXR1P and FXR2P. Absence of FMRP is thought to affect transport and/or translation of a subset of mRNAs and results in the formation of abnormal dendritic spines in patients and knock-out mice.
We have identified 4 novel proteins interacting with FMRP: NUFIP1, CYFIP1 and 2 (that share 95% of amino acids identity) and NUCIF1. Remarkably, CYFIP1 also interacts with Rac1, an important factor in neuronal maturation (1).
To study the physiological pathways in which FMRP and its partners are involved, we have chosen Drosophila, as its genome contains only a single FMR/FXR ortholog gene, dFMR (2), one dCYFIP gene and one dNUFIP gene. We found that in fly larvae brain, dFMR is specifically expressed in mushroom bodies, a structure involved in learning and memory. Analysis of the expression profile of dNUFIP and dCYFIP are in progress. We have generated dCYFIP null mutants and their phenotype is being analyzed. Preliminary results indicate a genetic interaction between dRac1 and dCYFIP mutants

1. Bardoni et al. (2001) Brain Bull. Res. 56: 375-382
2. Wan et al. (2000) MCB 20: 8536-8547

P0744. Towards an understanding of the Fragile X Syndrome: FMRP is translated at the synapses where it acts as a translational regulator

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The Fragile X syndrome is an X-linked disorder and the most common heritable form of mental retardation, and results from the deficit or absence of the FMRP protein which is expressed mainly in the brain and has been implicated in translational regulation.
While the vast majority of mRNAs is restricted to the cell soma of neuronal cells, a number of them are also transported into the dendrites, where they are translated. Transport and translation of specific mRNAs in extrasomal locations, plays an important role in nerve cell development and synaptic plasticity. Here we show by RT-PCR that *FMR1* mRNA that encodes FMRP is found in synaptoneurosomes. Furthermore, by electron microscopy studies we detected *FMR1* mRNA in proximal and distal dendrites of the hippocampus.
To identify potential localisation signal on the mRNA, we started by analysing the 3'UTR. We observed that the *FMR1* sequence contains several polyadenilation sites. DNA constructs were made expressing EGFP fused to the entire 3'UTR or to shorter region and transfected into mouse hippocampal neurons showing that alternative poly(A) sites are responsible for producing FMR1 mRNA molecules with different 3'UTRs differentially delivered into the cell. We have investigated the function of the FMRP protein at the synapses by analysing the translational efficiency of mRNAs in synaptosomal preparations. We find that some dendritic mRNA (a-CaMKII and Arc) are translated very efficiently in FMR1 K.O. mice as compared to wild type mice, whereas control RNA, (beta actin) is not affected showing that FMRP inhibits the translation of target mRNAs.

P0745. Differential gene expression in the fragile X mouse model

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Ten years of research showed that FMRP, the protein missing in fragile X patients, is an RNA binding protein that shuttles between the nucleus and cytoplasm. In neurons, the protein transports certain brain mRNAs towards the actively translating ribosomes near the synapses.
However, despite intense research it is still unclear why absence of the fragile X protein leads to the mental retardation, macroorchidism and specific behaviour problems observed in fragile X patients. In an attempt to unravel this mechanism, we performed gene expression analysis by means of the differential display method using the fragile X mouse model. In analogy to human patients, the fragile X knockout mouse shows a learning deficit and macroorchidism. The expression of approximately 95% of all genes in the hippocampus of control mice and fragile X knockout mice was compared. We isolated 224 sequences with a length range of 200-1100 bp. 143 sequences were underexpressed and 81 overexpressed in knockout mice. Using micro-arrays and real time PCR, for some of these sequences differential expression was confirmed. These partial cDNAs were sequenced, and homologies with known mouse and human ESTs and genes were searched on public and Celera databases.
The role of these genes differentially expressed in the fragile X animal model will now be investigated. These may help us to answer the question how the absence of fragile X protein relates to mental retardation in patients.

P0746. Quantitative analysis of DNA demethylation and trascriptional reactivation of the FMR1 gene in fragile X cells treated with 5-azadeoxycytidine

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The Fragile X syndrome is the leading cause of inherited mental retardation, affecting approximately one in 5000 individuals. In fragile X cells carrying a full mutation , hypermethylation of the expanded CGG repeat and of the upstream promoter leads to transcriptional silencing of the FMR1 gene. Absence of the FMR1 protein results in the phenotypic manifestation of the syndrome. We previously proved that treatment with 5-azadeoxycytidine of fragile X cell lines results in reactivation of the FMR1 gene restoring the production of the specific mRNA and protein product. We now show that this treatment causes demethylation of the FMR1 gene promoter. We employed the bisulphite sequencing technique to detect the methylation status of individual CpG sites in the entire promoter region upstream of the CGG repeat. Lymphoblastoid cell lines of fragile X males with full mutations of different sizes were tested before and after treatment with 5-azadeoxycytidine at various time points. We observed that individual clones are either completely demethylated or not, with few relevant exceptions. We also investigated the extent of methylation in the full mutation (CGG repeat) itself by Southern blot analysis after digestion with methylation-sensitive enzymes Fnu4HI and McrBC and found that the CGG repeat remains at least partially methylated in many clones with a demethylated promoter. This may explain the quantitative discrepancy between the large extent of promoter demethylation and the limited levels of FMR1 transcriptional reactivation estimated by quantitative real-time fluorescent RT-PCR analysis.
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P0747. Evidence for Skewed X Chromosome Inactivation in Females with the Fragile X Full Mutation

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X chromosome inactivation is generally thought to be a random occurence in somatic tissue. Skewed X-inactivation has been observed in several human disorders in which there is a deletion or mutation on one of the X chromosomes. This skewing is thought to be the result of selection against cells with growth disadvantage. Previous research by Rousseau et al. and Taylor et al. revealed a skewed X-inactivation pattern for females with the Fragile X full mutation.
As part of a larger Genotype-Phenotype research study, we analyzed molecular data for 51 females with the full mutation and 43 females with the premutation status for FXS, with an age range of 4 to 65 years. In contrast to females with the premutation, females with the full mutation demonstrated skewed X-inactivation with a tendency toward a higher activation ratio or proportion of normal active X. The median activation ratio for females with the full mutation was significantly larger than that of premutation carriers. Also, linear regression analysis revealed a significant positive relationship between the activation ratio and age for females with the full mutation, but not for females with the premutation. These results support previous research. In addition, activation ratio in women with a maternally inherited premutation was compared to women with a paternally inherited premutation. There was no signigant difference found between these two groups.

P0748. Loss of mutation at the FMR1 locus : a gene conversion?

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Fragile X syndrome is the most frequent inherited form of mental retardation. The mutation observed is almost exclusively an expanded (CGG) repeat in the first untranslated exon of the FMR1 gene. The repeat is polymorphic in length in the normal population (6 to 55 repeats) and may be unstable. Two types of mutations have been distinguished : premutation (up to 200 repeats) in the phenotypically normal carriers or full mutation (over 200 repeats) in the affected patients. In the latter case FMR1 is abnormally methylated and transcriptionally silent. Molecular diagnosis is based on the determination of the number of trinucleotide repeats by either Southern blot or fluorescent PCR. Transmission of the deleterious chromosome can also be established by haplotype analysis with flanking markers.
We have searched for the CGG expansion in a family comprising four siblings : two mentally retarded boys and their two sisters. Southern blot analysis revealed a large repeat expansion in the two boys and the oldest girl, whereas no expansion was observed in the youngest girl. These results were confirmed by PCR. Indirect analysis with three microsatellites (DXS548, FRAXAC1, FRAXAC2) allowed us to identify the maternal fragile X chromosome which is carried by the four children. In the youngest girl, the presence of this maternal fragile X chromosome is inconsistent with the absence of expansion. So, we discuss the hypothesis of a gene conversion where the segregation of flanking markers is dissociated from that of the CGG expansion.

P0749. Four novel mutations in the OFD1 (Cxorf5) gene in the Finnish patients with oral-facial-digital syndrome 1

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Oral-facial-digital syndrome type 1 (OFD1) is an X-linked dominant disorder characterized by malformations in face, oral cavity, and digits with a wide phenotypic variation. Recently, mutations in the OFD1 gene (Cxorf5) at Xp22 were found to underlie OFD1. We report here the identification of four novel mutations in the OFD1 gene in the Finnish families, two of which are familial and two sporadic.Three of the mutations in this study were located in the same exons as in the original study by Ferrante et al (Am J Hum Genet 2001;68:569-576). We also report the clinical findings of our patients. Our study confirms the causative role of the OFD1 gene in the pathogenesis of oral-facial-digital syndrome type 1.

P0750. Molecular basis of Oral-facial-digital type I (OFDI) syndrome

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Oral-facial-digital type 1 (OFD1) is part of the heterogeneous group of oral-facial-digital syndromes (OFDS). OFDI is an X-linked dominant condition lethal in males and is characterized by malformations of the face, oral cavity, and digits. Malformation of the brain and polycystic kidneys are commonly associated. By using a systematic mutation analysis approach we have identified the gene responsible for this genetic disorder, named OFDI, encoding a protein with unknown function. To gain insight into the pathogenesis of this disorder extensive mutation analysis and functional studies were undertaken. Twenty-seven OFDI patients were collected and mutations have been so far identified in 17 of them. Details on the newly identified mutations and on genotype/phenotype correlation will be presented. The presence of coiled-coil domains suggests that OFD1 may act via a protein-protein interaction mechanism. Interaction mating and two hybrid experiments are being performed to identify proteins potentially interacting with OFDI. Preliminary results show that OFDI does homo-interact through the central portion of the protein. Furthermore, subcellular localization experiments were performed on the wild type and mutated forms of the OFDI protein. Our study showed that the wild type protein concentrates in "cytoplasmic bodies" uniformly distributed in the cytoplasm while in the mutated forms, the cytoplasmic speckles become smaller, with a more diffuse distribution and tend to disappear. The functional characterization of the OFDI protein product will open the way towards understanding the molecular and cellular bases of OFDI as well as for the other forms of OFDs.

P0751. Partial deletion of the common 1,5 Mb critical region in an infant with classical Williams-Beuren syndrome.

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Williams-Beuren syndrome (WBS; OMIM 194050) is a contiguous gene deletion disorder with a variable clinical phenotype that is caused in most cases by a heterozygous microdeletion in 7q11.23. Due to two highly homologous flanking ~300 kb duplicons, the microdeletion is usually of similar size in almost all cases and encompasses a common ~1,5 Mb interval that contains at least 17 genes mostly of uncertain pathogenetic relevance. Phenotype-genotype correlation studies for WBS are hampered by the uniform size of the microdeletion.
Here we report the case of a 1-year-old boy with a full WBS-phenotype that is caused by a partial deletion of the common ~1.5 Mb interval. Initial analysis with two sets of commercially available FISH-probes (Appligene/Oncor and Vysis) yielded conflicting results. We therefore carried out deletion mapping with microsatellite markers and an array of targeted FISH probes. Our data could help to redefine the critical region for the full WBS phenotype (WBSCR). Evidence from two other cases in the literature also suggests that the centromeric portion of the 1,5 Mb interval is not always deleted in patients with full WBS-phenotype.
The conclusions from our work concern diagnosis and molecular aetiology of WBS:
1.) Quantitative differences in signal intensity after FISH analysis with commercial probes for a WBS microdeletion should be followed up carefully if partial deletions are not to be missed.
2.) Even in patients with full WBS-phenotype the underlying molecular defect is more variable than previously thought.

P0752. Characterisation of genes in the region of mouse chromosome 5 orthologous to the region deleted in Williams-Beuren Syndrome (7q11.23)and their human homologues.

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Williams-Beuren syndrome is a hereditary disorder caused by deletion of approximately 1.5Mb on human chromosome 7q11.23 and occurring in approximately 1/20000 live births. Symptoms include congenital heart disease, growth retardation, mild mental retardation with a distinctive cognitive profile and personality, facial dysmorphism and frequently infantile hypercalcaemia. The breakpoints of the commonly deleted region are flanked by highly homologous repeated regions of about 300kb. The flanking repeats are believed to mediate the disease causing deletion and have also caused difficulty in its genomic cloning and sequencing. The only aspect of the disease phenotye associated with a particular gene is the typical congenital heart defect, supravalvular aortic stenosis (SVAS) which is associated with deletion of the elastin gene. The orthologous region in mouse, on chromosome 5 (5G), does not have the flanking repeats, however the order of the genes within the commonly deleted region is conserved. Here we report expression profiles, gene structures and analysis of functional motifs for genes recently localised to the mouse equivalent of the commonly deleted region, including one gene, claudin13, which has not previously been reported to map to this region. Including these genes there are currently 29 transcripts shown to reside in this region of mouse chromosome 5. A comparison between mouse and human orthologues of the genes is also presented along with a complete physical map of the 1.4Mb mouse equivalent of the Williams syndrome region.

P0753. Identification of nine novel transcripts in the Williams-Beuren syndrome critical region

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Williams-Beuren syndrome (WBS) is a developmental disorder associated with haploinsufficiency of multiple genes at 7q11.23. Here, we report the characterization of WBSCR16, WBSCR17, WBSCR18, WBSCR20A, WBSCR20B, WBSCR20C, WBSCR21, WBSCR22 and WBSCR23, nine novel genes contained in the WBS commonly deleted region or its flanking sequences and of their murine orthologues. They were identified by mapping of previously undescribed human ESTs/cDNAs clusters to the WBS critical region. They encode an RCC1-like G exchanging factor, an N-acetylgalactos aminyltransferase, a DNAJ-like chaperone, NOL1/NOP2/sun domain-containing proteins, a methyltransferase, or proteins with no known homologies. Haploinsufficiency of these newly identified WBSCR genes may contribute to certain of the WBS phenotypical features.

P0754. Deletion breakpoint mapping in patients with Williams Syndrome using somatic cell hybrids

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Williams-Beuren syndrome (WS) is a developmental disorder caused by a hemizygous microdeletion of ~1.5Mb at chromosomal location 7q11.23. Up to 28 genes have been identified within the critical region. Hemizygosity for *ELN* causes the heart defect SVAS, but there is no clear evidence implicating any of the other genes in the aetiology of the syndrome. To aid genotype-phenotype correlations it is important to define the deletion breakpoints in patients with classic and partial WS phenotypes precisely. Homologous recombination between flanking repeats accounts for the high incidence of de novo deletions and the deletion breakpoints lie within these repeated regions in WS patients making them difficult to map. We have therefore made somatic cell hybrids (segregating the normal and deleted chromosome 7 homologues) from 30 patients with classic or partial WS phenotypes, designed a series of specific PCR primers using SNP technology, and mapped the breakpoints in the centromeric and telomeric repeat regions by PCR analysis of DNA from these cells. Our results indicate that the breakpoints cluster at two main loci at the centromeric end and at least three loci at the telomeric end. This has allowed us to group our patients according to their genotype for further detailed phenotypic analysis that includes complex cognitive testing.

P0755. Prader-Willi-like phenotype caused by multiple dosage of maternal 15q11-q13 region.

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Objective: Genetic diagnosis of Prader-Willi Sd. in a 13 years old male with normal motor development, obesity, moderate mental retardation, obsessive behaviour and small penis.
Methods: PWS methylation analysis was done by NotI-XbaI/pN09 hybridisation. Band densities were measured on a GS-700 Bio-Rad densitometer. Familial segregation analysis of seven 15q11-q13 linked markers was done by PCR. Karyotype was done by standard methods. Molecular cytogenetic studies included chromosome 15 painting using WCP15 probe and FISH analysis with probes LSI SRNPN/PML/CEP15 and LSI D15S10/PML/CEP15.
Results: Hybridisation showed the presence of both the paternal and maternal alleles, excluding a PWS methylation pattern. Maternal band intensity was 4 fold compared to normal controls. Familial segregation analysis of GABRA5 detected one paternal and two maternal alleles, suggesting a maternal trisomy of the region; D15S144 showed biparental inheritance. Karyotype: mosaicism 47,XY,+mar. Painting demonstrated the suspected chromosome 15 origin of the marker, which was present in 83% of metaphases. FISH results using specific probes were: 47,XY,+mar .ish der(15)(D15Z1++, D15S10++, SRNPN++). The marker showed the centromeric staining at both ends, and two adjacent signals for both SRNPN and D15S10 loci in the central part. The final cytogenetic result was mosaicism 47,XY,+ idic(15)(pter→q11.2 :: q11.2→pter).
Conclusions: The patient is a somatic mosaic for a marker chromosome of maternal origin which contains two inverted partial 15q11-q13 regions. Cells carrying the derivative chromosome are tetrasomic for genes contained in the region. The PWS-like features in the patient are caused by a multiple dosage of maternal 15q11-q13 genes.

P0756. Epsilon-sarcoglycan (SGCE), the gene mutated in myoclonus-dystonia syndrome, is imprinted.

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Myoclonus-dystonia syndrome (MDS, DYT11) has been defined as an autosomal-dominant disorder characterized by variable combinations of bilateral, alcohol-sensitive myoclonic jerks and dystonia. Using a positional cloning approach, we have recently identified heterozygous loss-of-function mutations in the gene for epsilon-sarcoglycan (SGCE) in MDS families. Pedigree analysis showed a marked difference in penetrance depending on the parental origin of the disease allele. This indicates a maternal imprinting mechanism, which has been demonstrated for the mouse orthologue. Bisulfite sequencing of the CpG-rich SGCE promotor showed a parent-specific methylation pattern in lymphoblasts. A rare single nucleotide polymorphism (SNP) in the promotor region allowed us to distinguish the parental alleles. The methylated strand showed the maternal polymorphism, while the paternal wildtype allele was unmethylated in all CpG dinucleotides examined.
Expression studies showed that SGCE is only paternally expressed in lymphoblasts.
Due to the fact that MDS is a non-degenerative central nervous system disorder we also investigated the promotor methylation in brain tissue. SNPs in the promotor region revealed that there is also differential methylation in human brain, suggesting regulation of expression in an allele-specific manner.
As imprinted genes are often located in clusters, we examined four adjacent genes by expression analyses in maternal and paternal UPD7 cDNA samples. SGCE and the adjacent PEG10 showed maternal imprinting in lymphoblastoid cell lines, whereas PP5 and BET1 were not imprinted. These results demonstrate that SGCE is also imprinted in humans and that chromosome 7q21 contains at least two imprinted genes.

P0757. Evaluation of a Mutation Screening Strategy for the Ube3a Gene in 33 Patients from 25 Families with Angelman Syndrome

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Angelman syndrome (AS) is a severe neurodevelopmental disorder which results from deficiencies of the maternal ubiquitin protein ligase 3A (UBE3A) gene caused by heterogeneous genetic alterations. This gene remains the only gene in the 15q11-q12 region found to play a role in the pathogenesis of AS. All UBE3A mutations reported so far were randomly distributed over the 2.6-kb major coding region including exons 8 to 16. The detection rates are around 30 % in non deletion/ non UPD/ non imprinting defect index cases, with an incidence of UBE3A point mutations in total AS patients estimated around 2-10 %. In this study, we investigated 33 patients from 25 families with a definite clinical diagnosis of AS and we evaluated 4 methods to establish a mutation screening strategy for the UBE3A gene. Automated SSCP analysis was used to screen the UBE3A gene for point mutations in all patients, in combination with direct sequencing if mutation could not be detected. Since the majority of UBE3A identified mutations are truncating mutations, we developed the protein truncation test (PTT) as a possible alternative approach to rapidly detect such inactivating alterations in this large gene. The combination of the different techniques allowed us to identify 22 point mutations from 25 families (88%)which represents the highest percentage of mutation reported so far in AS.

P0758. CREBBP mutations in 10 cases of Rubinstein-Taybi syndrome, including a mild variant showing a missense mutation

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Rubinstein-Taybi syndrome (RTS) is a well-defined autosomal dominant disorder of broad thumbs, broad halluces, short stature, microcephaly, facial anomalies and mental retardation. RTS can result from mutations of the gene for CREBBP. This nuclear protein controls chromatin structure (DNA accessibility) by acetylation of histones, and therefore assists in the initiation of DNA transcription. We report on 10 unrelated subjects, nine with RTS and one with a phenotype of possibly very mild RTS. Mutation analysis was performed using FISH and genomic sequencing. We identified five novel mutations (86del148nt, 1108C-T, IVS4+1G-A, IVS7+1G-A, 3524A-G), two gross deletions of the CREBBP gene and two single-nucleotide coding polymorphisms. The 1108C-T stop mutation was observed twice suggesting a mutational hotspot. The proband with the mild variant was very interesting. She presented a missense mutation (3524A-G) predicting a tyrosine-to-cysteine exchange (Y1175C) within a fully conserved (man vs. mouse) 79-aa segment of the protein domain that elsewhere was shown to confer the histone acetyltransferase activity. There has been one previous report of a CREBBP missense mutation in RTS (Murata et al., Hum Mol Genet 2001;10:1071-6). The “digito-facial” phenotype of this proband with clear-cut digital anomalies, subtle but typical facial changes, normal stature, normal head circumference and low but normal intelligence represents a mild RTS variant (“incomplete RTS”) that provides insight into phenotypic variation with RTS.

P0759. Molecular analysis of the CREBBP gene in 65 patients with Rubinstein-Taybi Syndrome.

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The Rubinstein-Taybi syndrome (RTS) is characterized by mental and growth retardation, broad thumbs, broad big toes and facial abnormalities. RTS is associated with mutations in the CREB-Binding protein (CREBBP) gene. Gross chromosomal rearrangements and microdeletions detected by fluorescence in situ hybridization and truncating mutations revealed by protein truncation test or Western blot analysis, account for only 20% of RTS cases. We report the use of molecular tools to thoroughly analyse the CREBBP gene in a cohort of 65 patients. These include cDNA probes to search for gross rearrangements by Southern blot analysis and to identify mRNA of abnormal size by Northern blot, intragenic microsatellite markers to look for intragenic deletions, as well as a complete set of primers to amplify each of the 31 exons of the gene for mutation search by direct sequencing. We analysed 62 patients and identified 29 mutations : 3 gross rearrangements by Southern blot and/or microsatellite analysis, 1 truncated RNA by Northern blot, 1 small intragenic deletion by RT-PCR and 24 point mutations resulting in either stop codons, aminoacid substitutions or abnormal splicing of the CREBBP RNA. Three additional patients were found to be deleted by FISH. Taken together, these results showed that the combination of the various techniques allowed us to identify a CREBBP mutation in 49.2% of RTS cases, which represents the highest percentage of CREBBP mutations reported sofar in RTS patients. These molecular tools will be useful to search for CREBBP mutations in other developmental pathologies with cancer predisposition and mental retardation.

P 16. Molecular Basis of Development

P0760. Expression pattern of the RSK2- or Coffin-Lowry syndrome gene during murine development

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Mutations in the RSK2 gene on Xp22.2 are causing Coffin-Lowry syndrome, which is characterized by severe psychomotor retardation, facial and digital dysmorphism, and progressive skeletal deformations affecting mostly the vertebral column. About 50-60% of the mutations identified so far lead to premature translation termination and are predicted to cause loss of function alleles. Some missense mutations are associated with milder phenotypes. In one family, the R383W mutation is causing non-syndromic X-linked mental retardation without other symptoms. RSK2 is a member of the 90kDa ribosomal-S6-serine/threonine kinase family acting at the distal end of the Ras signalling cascade. After activation by MAPKs, RSK2 translocates to the nucleus and regulates gene expression by phosphorylation of transcription factors such as CREB. RSK2 knock-out mice are 10% smaller compared to wildtype littermates. Additionally, RSK2 KO mice have impaired learning and poor coordination, suggesting

that RSK2 seems to have similar roles in mental functioning both in mice and humans. To investigate the spatio-temporal expression spectrum of RSK2 during mouse development, we performed RNA in situ hybridization. In early embryonic development (ED 9.5-10.5) high RSK2 expression was observed exclusively in somites and lateral plate mesoderm from which among other tissues the vertebral column develops. At later embryonic stages (ED 12.5-14.5) enhanced RSK2 mRNA levels are detected in the peripheral nervous system (dorsal root ganglia) and in sensory ganglia of the cranial nerves. In contrast to the more widespread expression in multiple tissues of adult mice, RSK2 shows a highly specific expression spectrum during embryonic development.

P0761. HoxB1 allelic variants in hindbrain malformations

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Chiari complex is the most frequent pathology among hindbrain malformations, characterized by caudal cerebellar herniation, sometimes associated with lower brain stem dysmorphism, skull bases and vertebral anomalies. In vertebrates hindbrain, generation of regional diversity is achieved through a segmentation process that, during primary neurulation, leads to the formation of 7 metameric units, called rhombomeres. In this process, Hox genes display a key role in controlling and regulating neuronal migration and in mainteining cellular segmental identity. Among labial hortologous, Hoxb-1 gene is the first one to be activated in CNS and the only one to show an expression domain restricted to rhombomere 4 and in the neural crest cells that from r4 migrate in the second branchial arch. We performed the mutational screening of the homologous HOX-B1 gene in 49 patients and 103 control individuals. Sequencing of abnormal SSCP conformers revealed the existence of three allelic variants characterized by the presence of several in cis associated mutations, all affecting the NH2 terminal region of the gene. α1 haplotype is characterized by the presence of two synonimous transitions (C237T and G450A) and one missence mutation (A309T); α2 variant shows, in addition, a 9-bp tandem duplication (CCCACAGCG) at position +80, while α3 presents three silent substitutions (G114A, C213T and G246A) and one missense mutation (C167T). Since the different distribution of the α1 and α2 haplotypes in controls and patients and the absence of α3 variant in the first population, we hypotize these mutations as predisposing genetic factors for the insorgence of pathology.

P0762. Vax2 inactivation in mouse determines alteration of the eye dorsal-ventral axis, misrouting of the optic fibers and eye coloboma

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Vax2 is a homeobox gene whose expression is confined to the ventral portion of the prospective neural retina. Overexpression of this gene at early stages of development in Xenopus and in chicken embryos determines a ventralization of the retina, thus suggesting its role in the molecular pathway underlying eye development. We have generated and characterized a mouse with a targeted null mutation of the Vax2 gene. Vax2 homozygous mutant mice display incomplete closure of the optic fissure that leads to eye coloboma. This phenotype is not fully penetrant suggesting that additional factors contribute to its generation. Vax2 inactivation determines dorsalization of the expression of mid-late (EphB2 and ephrin-B2) but not early (Pax2 and Tbx5) markers of dorsal-ventral polarity in the developing retina. Finally, Vax2 mutant mice exhibit abnormal projections of ventral retinal ganglion cells. In particular, we observed the almost complete absence of ipsilaterally projecting retinal ganglion cells axons in the optic chiasm and alteration of the retinocollicular projections. All these findings indicate that Vax2 is required for the proper closure of the optic fissure, for the establishment of a physiological asymmetry on the dorsal-ventral axis of the eye and for the formation of appropriate retinocollicular connections.

P0763. Search for somatic 22q11.2 deletions in patients with conotruncal heart defects

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The wide range of clinical variability in patients with 22q11.2 deletions has been demonstrated in numerous studies. Nevertheless, it is still an open question if major genetic factors contribute to clinical expression. Therefore one aim of this study was to investigate, if patients with 22q11.2 deletion and conotruncal heart defects show a “second hit” somatic 22q11.2 deletion in tissue from the conotruncus, heart vessels or thymus. The second aim was to analyse patients with conotruncal heart defects without 22q11.2 deletion in blood cells for somatic deletion mosaicism. Parents of 19 patients with conotruncal heart defects (IAA, TAC, pulmonary atresia with VSD) agreed to collect and study somatic tissue from heart surgery in their children. 5 of these 19 patients had 22q11 deletions shown by FISH analysis on metaphase spreads from peripheral lymphocytes with 10 DNA probes from the DGS1 region. DNA was prepared from thymus and/or heart vessels and/or conotruncus tissue and peripheral lymphocytes in each patient and analysed with 18 microsatellite markers from the DGS1 region for allelic loss. Results did not show any allelic loss, thus there was no evidence for a somatic 22q11.2 deletion. Therefore somatic 22q11.2 deletions apparently do not play a major role in conotruncal heart defects in patients with or without germ line 22q11.2 deletion.

P0764. Expression profiling in mouse neural development and differentiation.

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Our approach combines gene array expression technology and murine subtractive cDNA library to isolate unique and specific genes preferentially expressed in the embryonic telencephalon. We have randomly sequenced 3600 cDNA clones (ESTs) from a cDNA subtractive library. A set of unique transcripts (1026) have been identified, selected and arrayed on glass coated slides. A series of experiments based on the potential of cell lines (P19, neuro2A, PC12) to be induced to differentiation into specific neuronal cell subtypes by Retinoic Acid (RA) or Neural Growth factor (NGF), are undergoing. This will permit the isolation of genes that are differentially expressed before and after neuronal “in vitro”differentiation. The results will be confirmed by Real time PCR assays. A detailed sequence analysis of the 372 identified cDNA clones was performed using public domain DataBases (such as dbEST, Unigene, Homologene, Locus Link and OMIM), to verify the quality of the library, to identify the human homologs, and to map and correlate them to neurological disorders. In particular 20% of the selected clones have no public database match to date, and 23% correspond to genes with unknown function. To determine the spatio-temporal expression profile of 110 cDNAs, we have performed in-situ mRNA hybridization on mouse embryos (sagittal and coronal sections of E14.5 embryos and whole-mount E10.5 embryos) and adult brains. Moreover, experiments are undergoing for testing the value of this cDNA array in order to unravel the molecular defects of the developing brain of mice models mutated in the Tbr1 and Lis1 genes.

P0765. Expression of SMADIP1 during early human development correlates with the phenotype of a syndromic form of Hirschsprung disease

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The smad binding protein 1 gene (SMADIP1, MIM 605802) has been identified as causing a polytopic embryonic defect (MIM 235730) including midline anomalies (agenesis of the corpus callosum,

congenital cardiac defect, hypospadias), facial dysmorphism, mental retardation and enteric nervous system malformation (Hirschsprung disease, HSCR). We recently screened the SMADIP1 locus in a series of 19 unrelated patients with this phenotype and identified de novo large-scale SMADIP1 deletions or truncating mutations in 8 cases. To further investigate the role of SMADIP1 during embryogenesis, we performed RNA in situ hybridization at early stages of human development. According with HSCR and facial dysmorphism in patients, SMADIP1 is expressed in the enteric nervous system and other neural crest derived cells (peripheric nervous system, facial neurectoderm and cranial nerve ganglia). SMADIP1 mRNAs are also detected in the central nervous system as soon as day 33 (carnegie 15). In agreement with other clinical features (hypospadias, strabismus, limbs and kidney anomalies, and hypotonia) SMADIP1 is further expressed in genital tubercle, developing eye, limbs, kidney and muscles. Although congenital cardiac defects are frequently observed, no SMADIP1 expression is detected in the developing heart. However, this expression pattern correlates with the spectrum of malformations observed in patients and confirms the pleiotropic role of SMADIP1 during human development.

P 17. Molecular Basis of Mendelian Disorders

P0766. Genotype Analysis of the NF1 Gene in the French Canadians From the Québec Population

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder that afflicts about 1 in 3,500 individuals. The NF1 gene spans about 350 kb at 17q11.2 with 60 exons and has a high mutation rate leading to 50% sporadic cases. We genotyped 19 NF1 families including 85 individuals (45 affected and 40 unaffected) from the French Canadians of the Québec population and investigated deletion mutations, allele frequency distribution, linkage and linkage disequilibrium (LD) by using six intragenic polymorphic markers including 2 RFLPs (EcoRI and RsaI) and 4 microsatellites (IVS26-2.3, IVS27AC28.4, IVS27AC33.1, and IVS38GT53.0) which are distributed along an approximately 65kb of the gene. Genotype analysis indicated families 7610 and 7473 bear unusual deletions. In Family 7610 the deletion removed the entire NF1 gene except exons1 to 4b. The breakpoint of the deletion is located between exons 4a and 4b. The deletion 7473 was derived from the maternal chromosome and exons 1 to 5 were deleted. The breakpoint of the deletion is located between exons 7 and 13. Clinical manifestations were mild suggesting that deleting either upstream or downstream contiguous sequence may not lead to a severe phenotype. Deletion of both upstream and downstream sequences on both sides of the NF1 gene may be needed to cause severe clinical features. The allele frequencies of microsatellites IVS27AC28.4 and IVS38GT 53.0 are compared to previously reported data from Caucasians, including Spanish and Italians. The difference is statistically significant ($P<0.0036$) for marker IVS27AC 28.4 between the Québec French Canadian and the Italian population.

P0767. Mutation Analysis of the EXT Genes in Taiwanese Patients with Hereditary Multiple Exostoses

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Hereditary multiple exostoses (HME) is an autosomal dominant disorder characterized by short stature , cartilaginous excrescences near the ends of the diaphyses of the bones of the extremities,and increased risk of chondrosarcoma . Three chromosomal loci have been comprised in this genetically heterozygous disease: EXT1 gene on chromosome 8q23-q24, EXT2 on 11p11-p13, and EXT3 on 19p. Both the EXT1 and EXT2 genes had been cloned and been defined as a new family of potential tumor suppressor genes. We had analyzed five patients with clinical features of multiple exostoses, of which one is sporadic and four are familial cases. For determining the mutant spectrum of disease attributable to abnormalities in the three EXT loci, linkage studies were performed before the mutation analysis. The results showed that one family linked to EXT1 locus and three linked to EXT2 locus. Four novel mutations were identified: a frameshift mutation (K218fsX247) and a nonsense mutation (Y468X) in EXT1 gene; a missense mutation (R223P) and a nonsense mutation (Y394X) in EXT2 gene. Moreover, according to the definite disease-causing allele, linkage analysis provides a reliable clue and could be utilized as an alternative approach for clinical and prenatal diagnosis.

P0768. Evidence Of The Existence Of At Least A Fourth Locus For ADNFLE

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), characterised by clusters of nocturnal seizures with a frontal lobe semiology, mostly occurring during non-REM sleep, follows autosomal dominant inheritance with incomplete penetrance (70-80%). Three loci have been associated to this syndrome: ENFL1 (20q13.2), ENFL2 (15q24) and ENFL3 (1q21). Three mutations responsible for ADNFLE have been reported in the CHRNA4 gene (ENFL1 locus), coding for the alpha4 subunit of the neuronal nicotinic acetylcholine receptor (nAChR) and two mutations have been found in the CHRNB2 gene (ENFL3 locus), coding for the beta2 subunit of the same receptor. However, the identified mutations account for a minority of ADNFLE cases. Additional brain-expressed nAChR subunit genes (alpha2-7 and beta2-4) are candidates for ADNFLE. We performed linkage analyses to evaluate the association between ADNFLE and alpha2-7 and beta2-4 subunits in four families. Six chromosome regions were analysed: 1q21 (CHRNB2), 8p21 (CHRNA2), 8p11.2 (CHRNA6 and CHRNB3), 15q14 (CHRNA7), 15q24 (CHRNA5/A3/B4) and 20q13.2 (CHRNA4). Significantly negative LOD score values were obtained in each family, except in two cases (CHRNA4, family 32; CHRNA2, family 10), where, however, no mutations were detected by sequence analysis. Besides further supporting locus heterogeneity of the disease, these findings exclude the involvement of all known neuronal brain-expressed nAChR subunits in the etiopathogenesis of ADNFLE in the analysed families and demonstrate the existence of at least a fourth locus, probably not belonging to the nAChR gene family, involved in this syndrome.

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P0769. Molecular and genetical study of phenylketonuria in Ukraine

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We carrier out genetic investigation of 100 patients with clinically diagnosed phenylketonuria in order to identify PAH gene mutations. Using DGGE and dHPLC techniques we have screened mutant alleles in exons 1, 3, 7, 11, 12 of PAH gene. Identification of mutations were performed by RFLP and direct DNA sequencing. The most frequent mutation found according to our study was R408W (57 %). The frequency of other mutations were: R158Q – 3,5%, R252W – 2,9%, P281L – 2,3%, Y414C – 1,5%, Ivs10nt546, Ivs12nt1, R261Q, G272X, S273F, R413P – 0,6-1%. PAH gene STR and VNTR polymorphisms analysis were performed in this group of patients too. PKU is a highly heterogeneous trait showing a broad continuum of phenotypes. On the basis of individual data on phenylalanine tolerance and pretreatment phenylalanine serum the 24 patients were assigned to one of the four arbitrary phenotype categories: classic PKU, moderate PKU, mild PKU and MNH. 19 patients with genotypes R408W/R408W (7 patients), R408W/R158Q (2), R408W/R261Q (1), R408W/Ivs10nt546 (1), R408W/Ivs12nt1 (1), S273F/R413P (1), R252W/x (1), R408W/x (1), x/x (4) had classic PKU. 3 patients with genotypes R408W/P281L, R261Q/x, x/x were diagnosed as moderate PKU and 2 patients (genotypes R158Q/x and x/x) had mild PKU. It is interesting to note that two untreated patients with genotype R408W/Y414C were diagnosed as classic PKU and MHP basing on clinical date (they are 11 and 7 years old). This date confirm the necessity of treatment for PKU patients with mild PAH gene mutations.

P0770. A new CDMP 1 mutation in a family with brachydactyly type C

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Inherited isolated brachydactyly type C (OMIM 113100) is an autosomal dominant disorder with marked variability. Type C consists of shortness of 1st metacarpal, 2nd, 3rd and 5th middle phalanges, hypersegmentation of the proximal phalanges of the 2nd and 3rd digits and ulnar deflection of the index. In most families, the anomalies are restricted to the hands. Associated findings such as short stature, radio-ulnar and humero-radial abnormalities, wedging of vertebrae, hip dysplasia, epiphyseal changes, foot anomalies and cupped ears have also been reported.
Locus heterogeneity for brachydactyly type C has been demonstrated. In a kindred with anomalies of upper and lower limbs reported by Haws (1963), a gene has been localized in 12q24 (Polymeropoulos et al., 1996); brachydactyly limited to the hands was mapped to chromosome 20q11.2 (Lin et al., 1996). Several mutations in the morphogen CDMP1, a member of TGF-b superfamily mapping to 20q, were predicted to cause haploinsufficiency (Polinkovsky et al., 1997). We present the clinical findings of 4 affected members of a 3-generation family with involvement of upper limbs only. Blood was obtained from 8 members of the family. Haplotype analysis showed segregation of the disease phenotype with markers on chromosome 20q11.2, but not on chromosome 12q24. Subsequently, mutation analysis by sequencing showed a t1380c transition resulting in a F354S mutation in CDMP1. This mutation segregated in the 4 affected patients and was never observed in normal members of the family, nor in 50 controls. As far as we know, this mutation has never been described before.

P0771. DGGE analysis of the low density lipoprotein receptor gene mutations in patients with familial hypercholesterolemia in Greece

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We used the denaturing gradient gel electrophoresis (DGGE) method to investigate 45 Greek patients with familial hypercholesterolemia (FH) for mutations in the promoter region and the 18 exons and their flanking intron sequence of the low density lipoprotein (LDL) receptor gene. Eight aberrant DGGE patterns were found, and the underlying mutations were characterized by DNA sequencing. These mutations were located in 6 different exons (exons 2, 6, 8, 9, 12, 14). Among them 8 were missense mutations (C6W, S265R, A370T, Q363P, Q363X, D365E, V408M, G571E) and 1 was a splice defect (2140+5G>A). The splice site mutation and the Q363P are detected for the first time in the Greek population. The prepositus for the splice defect was also double mutant for the mutations Q363X and D365E previously found in Greek-Cypriot subjects. These identified mutations co-segregated in their family members with defective LDL receptor activity and hypercholesterolemia, and are thought to be causal for the FH phenotype since these were the only molecular defects identified in the entire region and splice site consensus sequences. These results demonstrate that there is a broad spectrum of mutations in the LDL receptor gene in the Greek population

P0772. Mutation and haplotype analysis of ABCA4 in mixed Spanish families and implication of this gene in a pattern dystrophy phenotype.

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Studies of genotype-phenotype correlations highlighted the function of ABCA4 in retinitis pigmentosa (RP), cone-rod dystrophy (CRD) and Stargardt disease (STGD). Initial screening of ABCA4 variants showed a correlation between the type of mutation and the severity of the disease. Later, in vitro studies of several recombinant ABCR mutants revealed a wide range of severity of biochemical defects [Sun et al., 2000]. In the present study we have undertaken mutational and haplotype analysis of ABCA4 in three mixed pedigrees segregating different retinal dystrophies to identify the combination and type of mutations causing the diseases. In family I, we have shown cosegregation of different ABCA4 alleles with STGD, FFM, CRD and even pattern dystrophy simulating FFM (FFM-like PD). To our knowledge, this is the first report of a PD phenotype explained by mutations in ABCA4. Based on the fact that this disease is milder than STGD, it is tempting to speculate that it may be explained by a combination of two mild alleles. On the other hand, in family II, segregating STGD and RP phenotypes, the involvement of ABCA4 in STGD is clear, but this is not the case for RP. Finally, in family III, also segregating STGD and RP, ABCA4 fails to explain either phenotype. Our data highlight the wide allelic heterogeneity involving this gene and support the genetic heterogeneity (beyond ABCA4) of mixed STGD/RP pedigrees.

P0773. Did neuroferritinopathy originate in France?

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We have identified a new dominant neurodegenerative disease, neuroferritinopathy, which results from a pathological mutation in the ferritin light chain gene on chromosome 19 (OMIM: 606159). A single adenine insertion in exon 4 disrupts the DE loop and E helix at the carboxy-terminal end of the subunit and leads to brain iron accumulation and a variable clinical phenotype that can mimic Huntington's disease, parkinsonism and dystonia. Several cases have now been recognised in the UK and share a common haplotype. We have now identified the same mutation in a French family, previously described in the literature with an atypical dystonia, MRI evidence of basal ganglia degeneration and reduced activities of several mitochondrial respiratory enzymes (Caparros-Lefebvre et al 1997 J Neurol Neurosurg Psychiatry, 63: 196-203). We show that this family shares the closest marker in the disease haplotype found in north England. Genealogical research has linked some of the English families to the 18th century Coulthard family of North Cumbria. The surname has been traced to a Norman extraction based on the town of Coudehard raising the possibility of a much more ancient origin for the mutation. Further genealogical and haplotype analysis will clarify the degree to which this mutation should be sought in French people with atypical late onset neurological basal ganglia dysfunction.

P0774. A Novel *AVPR2* Mutation in a Kindred with Nephrogenic Diabetes Insipidus

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Nephrogenic diabetes insipidus (NDI) is a congenital disorder characterised by the excretion of large volumes of diluted urine due to impaired renal concentration in response to the hormone arginine vasopressin (AVP). Most cases are inherited in an X-linked recessive manner and present mutations in the V2 vasopressin receptor (*AVPR2*) gene. A few cases are autosomal recessive, due to mutations in the AVP-sensitive water channel gene, aquaporin-2 (*AQP2*).
A female index case presented with an average fluid intake of 12L/day. An additional four male subjects were affected, presenting an inheritance suggestive of an X-linked disorder. Clinical and biochemical analysis of the index case showed incomplete responses to the water deprivation and vasopressin loading tests. Partial hemodynamic and coagulation responses to the synthetic V2-specific agonist dDAVP were observed. Genetic analysis was performed by PCR amplification of the coding regions and exon/intron boundaries of the *AVPR2* gene, followed by automated DNA sequencing.
A 493G→C transversion in exon 2 was observed, leading to the substitution of an alanine by a proline at codon 165. Restriction enzyme analysis allowed confirmation of the mutation and its cosegregation with the disease. In addition, analysis of 105 alleles from 70 unrelated individuals revealed an absence of this abnormality.

Our study has, therefore, identified a previously unreported mutation of the *AVPR2* gene, affecting a transmembrane domain of the receptor. The marked degree of NDI symptoms exhibited by the heterozygote female patient, in spite of her partial responses to stimulation tests, could be explained by a mechanism of skewed X-inactivation.

P0775. Mutation in the gene for protein tyrosine phosphatase SHP-2 (*PTPN11*) in a large family with Noonan/cardio-facio-cutaneous syndrome

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Noonan syndrome (NS, MIM 163950) is an autosomal dominant condition characterised by facial dysmorphy, congenital cardiac defects and short stature. Most of the clinical features of Noonan syndrome overlap with cardio-facio-cutaneous (CFC) syndrome. In CFC syndrome patients are more severely affected with specific skin abnormalities and moderate mental retardation. Recently, a gene responsible for Noonan syndrome in some families has been cloned. Missense mutations in *PTPN11*, the gene encoding the non-receptor protein tyrosine phosphatase SHP-2, is responsible for at least 50% of the Noonan syndrome cases.
A large, four generation Belgian family with NS in some and CFC syndrome in other family members, was previously used to fine map the Noonan syndrome candidate region in 12q. We now report the identification of a mutation (Gln79Arg) in the *PTPN11* gene in this large family. The mutation was found in both the CFC and NS individuals from this family. We believe that CFC syndrome is a heterogeneous condition (as well as NS) and that some CFC syndrome cases may be the result of variable expression of an SHP-2 mutation. Screening of a larger group of Noonan syndrome and CFC syndrome patients is in progress.

P0776. High Prevalence of Molecularly Defined Long QT Syndrome in Finland

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Long QT syndrome (LQTS) manifests as prolonged QT interval on ECG, ventricular arrhythmias and risk of sudden death. The prevalence of inherited LQTS is estimated as 1:10 000. Mutations of five ion channel genes (KCNQ1, HERG, KCNE1, KCNE2 or SCN5A) may cause LQTS. We have identified two founder mutations (KCNQ1-Fin and HERG-Fin) that together account for 35-40% of LQTS cases in the genetically isolated Finnish population.
This study was conducted to approximate the prevalence of inherited LQTS in the Finns and, in particular, to screen for mutations of SCN5A among LQTS probands. The causal mutation was documented in 86/236 (36%) families studied, and 631 mutation carriers were identified corresponding to a population prevalence of 1:8000 (Table 1). Case detection rate suggests that the actual prevalence is at least 1:5000. Simple PCR assays for the KCNQ1-Fin and HERG-Fin mutations alone detect 35% of cases. Samples from 150 LQTS probands without prior mutation detection were screened for SCN5A mutations. One amino acid change (G5851T corresponding to V1951L) and two silent polymorphisms (G4218A and C5457T) were detected.
In conclusion, DNA analyses show great promise in establishment of the LQTS diagnosis and may identify variants showing a disease-modifying role. Inherited LQTS may be more prevalent in Finland than many other Western populations. The LQT1, LQT2 and LQT3 subtypes of LQTS occur in relative frequencies of 75%:21%:4%, respectively.

Table 1.

LQTS type	Mutation carriers	Families	Mutation
KCNQ1	475	66	6
KCNQ1-Fin	428	59	
HERG	133	19	9
HERG-Fin	71	9	
SCN5A	23	1	1
All LQTS patients	631	86	16

P0777. PAH gene mutations identified in Lithuania

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We report the spectrum of the PAH gene mutations in patients with phenylketonuria residing in Lithuania. A total of 184 independent PAH chromosomes were investigated. All 13 exons of the PAH gene of all PKU probands were scanned for DNA alterations by denaturing gradient gel electrophoresis (DGGE). In the cases of a specific DGGE pattern was recognized, mutations were identified by direct fluorescent automated sequencing or by restriction enzyme digestion analysis. 19 different PAH gene mutations were identified on 173 PKU chromosomes (95%). The most common ones were R408W (73.6% chromosomes) and R158Q (6.6% chromosomes) whereas the remaining mutations appeared to be rare (relative frequencies were from 0.55% to 2.2%.). In 11 PKU chromosomes for 9 patients repeated DGGE scanning of the whole coding region of the PAH gene and sequencing exons if specific pattern was recognized revealed no mutations. Most likely, in these cases mutant chromosomes may harbor large deletions or intronic splice mutations in the PAH locus.
52 individuals with PKU were found to be homozygous for the PAH gene mutation. The vast majority of such patients (51 or 57%) appeared to be homozygous for R408W (PAH genotype R408W/R408W), while the proband in one family was homozygous for R158Q (PAH genotype R158Q/R158Q). 33 (36%) patients with PKU were compound heterozygous: in 31 (33%) cases R408W and a rare mutation were identified and in two cases both mutations were rare.

P0778. Mutations of the CYP11A gene could not explain all cases of congenital lipoid adrenal hyperplasia without StAR gene mutation in our Caucasian population.

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Congenital lipoid adrenal hyperplasia (lipoid CAH) is a rare autosomic recessive disorder affecting the first step of steroid biosynthesis, leading to the absence of glucocorticoids, mineralocorticoids and sex steroids. Affected individuals present with salt loss, dehydration and patients with XY genotype are phenotypically females. In contrast with other CAH, this disease is not caused by a defect in the enzyme (P450scc), but in the Steroidogenic Acute regulatory protein (StAR), implicated in the transport of cholesterol from the outer to the inner mitochondrial membrane where P450scc is localized. Almost all lipoid CAH patients (41/42) who are not of Caucasian origin are homozygous or compound heterozygous for mutations of the StAR gene. By contrast, in our 11 unrelated Caucasian families, we have found only 6 families with mutations of the StAR gene. In the 5 other ones, StAR mRNA and Dax-1 and SF-1 genes, of which mutations cause adrenal insufficiency, have been sequenced and appear to be normal. As a de novo heterozygous mutation of the CYP11A gene encoding P450scc has recently been reported to cause lipoid CAH, we have sequenced this gene and found mutations in two patients. This study shows that mutations of the StAR gene are responsible for lipoid CAH in only 60% of patients of Caucasian origin. Compound heterozygous mutations of the CYP11A gene can also be responsible if residual P450scc enzymatic activity produces enough progesterone to avoid spontaneous abortion. Nevertheless, other genes should be involved to explain the genetic lesion in our 3 remaining families.

P0779. CTG repeat instability in human DM1 germ cells.

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Myotonic dystrophy (DM1) is caused by the expansion of an unstable CTG repeat located in the 3'-untranslated region of the DM gene (DMPK). The number of CTG repeats is polymorphic in the general population a ranging from 5 to 37 repeats. DM1 patients have expansions of greater than 50 repeats and up to many thousands. The size of the repeat is positively correlated with the severity

of the disease and inversely correlated with the age of onset of symptoms.Dramatic instability with very large intergenerational increases and contractions is observed in DM1 patients.Detailed studies of somatic mosaicism have revealed that it is tissue specific, biased toward further expansion and continuous throughout the life of an individual.The trinucleotide repeats instability mechanisms involved in DM1 are unknown.In order to gain a better understanding of the dynamics of repeat instability in the male germline, we have used sensitive small pool-PCR analyses (SP-PCR) to compare blood and sperm DNA from 23 males, with different age, CTG repeat expansion and clinical form.Analysis of sperm DNA from control individuals showed that small normal alleles were stable. Sperm samples of DM1 patients revealed both different levels of mosaicism and patterns of distribution for the expanded allele, designing a characteristic pattern in each clinical group. The comparison of these results with those obtained from peripheral blood will allow us to define, in accordance with age and clinical form, specific patterns of mosaicism in somatic and germline cells for DM1 individuals.

P0780. Mutation analysis of PAH gene among Latvian patients

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Phenylketonuria (PKU), an autosomal recessive genetic disorder, is caused by a deficiency of the hepatic phenylalanine hydroxylase enzyme (PAH). Sixty patients were detected from 1980 to 2001; the approximate incidence of PKU is 1 of 8700 new-borns in Latvia. Fifty patients from 48 unrelated families were screened for the presence of six PAH gene mutations: R408W, R261Q, R252W, G272X R158Q and IVS10nt546 using ASO and PCR/RED methods. The mutation detection rate was 86,0% among the studied alleles. Out of 100 alleles under study, 77 (77%) were identified as defective due to R408W. Twenty-eight patients were homozygotes for R408W, 21 were compound heterozygotes for R408W. Other five mutations had a very low incidence of PKU alleles: R158Q -4%, R261Q - 2%, G272X - 1%, R252W - 1%, IVS10nt - 1%. In 14 cases (14%) probands were compound heterozygotes for different PAH locus mutations, one of them was unidentified. The location of unknown mutations was found using the DGGE method. DNA sequence analysis of the exons which showed positive DGGE signals found out 4 different mutations: E280K (5%), A104D (2%), E178G (1%), P281L (1%). The sequence analysis of second allele for other chromosomes is in progress.
Presence of severe R408W mutation in 77% of PKU alleles explains the high rate of PKU patients with severe phenotype.

P0781. Highly skewed X-inactivation pattern in a female with unique presentation of hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency.

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Partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) is an X-linked recessive disorder of purine metabolism characterised with onset of gout and/or urolitiasis often in adolescence. This phenotype occurs almost exclusively in males. We follow a girl who presents gouty arthritis of big toe and hyperuricaemia from the age of 9 years. Normal activity of phosphoribosylpyrophosphate synthetase, loss of HPRT activity, raised adenine phosphoribosyltransferase activity and raised nicotinamide adenine dinucleotide concentration in erythrocytes revealed partial HPRT deficiency. The loss of HPRT activity was found also in the patient's father, who presented with renal colics and gout since the age of 18 years. The sister of our female patient is asymptomatic and her HPRT activity is within normal limits.
Mutation analysis revealed that both sisters inherited from their father a previously described mutation in the 3rd exon of HPRT gene, c.158T>C (V53A). X-inactivation study performed in peripheral blood leukocytes showed that the X-inactivation is highly skewed in both sisters leaving the paternal mutated chromosome predominantly active in the symptomatic girl, while the second asymptomatic girl has almost exclusively active maternal non-affected chromosome.
Conclusions: 1. The unusual description of phenotype shows a possibility of presence of partial HPRT deficiency in girls with unexplained hyperuriceamia.
2. Results of biochemical tests performed in heterozygotes may fail because of X-inactivation status. Mutation analysis is necessary for a reliable identification of carriers of mutated gene.
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P0782. Paternal inheritance of mtDNA in a patient with mitochondrial myopathy

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Mitochondrial DNA (mtDNA) is thought to be strictly maternally inherited in mammalian species: Sperm mitochondria disappear in early embryogenesis, either through selective destruction, inactivation or by simple dilution due to the surplus of oocyte mitochondria.
A very small amount of paternally inherited mtDNA has been detected by PCR, identified after several generations of interspecific backcrosses. However, recent studies using microinjected sperm into mouse oocytes support the hypothesis that sperm mitochondria are targeted for destruction by nuclear-encoded proteins, but the underlying mechanism remains a mystery. In this report we show for the first time that paternally derived mtDNA can indeed survive and contribute substantially to the mtDNA pool in man. In a patient with mitochondrial myopathy due to a novel two base pair (bp) deletion in the ND2 gene, 90% of the muscle mtDNA was paternal in origin. The two mtDNA haplotypes were different at 18 positions. The mtDNA haplotype in muscle was identical to the haplotype found in the father, while that of blood was identical to the mtDNA haplotype in the mother. This phenomenon may be more common than generally believed because mitochondrial haplotypes are often not investigated and because substantial differences are required in order to distuguise the haplotypes in a routine analysis.

P0783. Haploinsufficiency of DYRK1A on chromosome 21q22.2 is associated with microcephaly

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Recent studies of primary microcephaly are frequently based on homozygosity mapping in consanguineous families and have revealed 5 loci for recessive forms of microcephaly, but so far no gene has been identified by this approach. Examination of balanced translocations is an alternative approach to identify genes involved in microcephaly. We report on a 12-year old girl with prenatal onset of microcephaly, a severe developmental delay including speech defect and seizures. Structural brain malformations were excluded by MRI. Chromosome analysis revealed an apparently balanced translocation t(2;21)(q22;q22). Molecular cytogenetic techniques were used to find breakpoint-spanning clones. Subsequent breakpoint analysis showed that the chromosome rearrangement led to disruption of the DYRK1A gene. Semiquantitative RT-PCR experiments revealed a 50% reduction of DYRK1A expression in the patient's lymphoblastoid cell line compared to a control cell line. The chromosome 2 breakpoint maps within the putative tumor suppressor LRP1B, a member of the low density lipoprotein receptor family.
Dyrk-related kinases belong to a new family of protein kinases that have been suggested to be involved in the regulation of cellular growth and development. The Drosophila homolog minibrain (mnb) is required for normal postembryonic neurogenesis and mutations in this gene lead to size reduction of the brain. We could show that haploinsufficiency of DYRK1A is associated with microcephaly. This is supported by the finding of microcephaly as a main feature in patients with monosomy 21q22. We would suggest that other patients with primary microcephaly should be screened for mutations in the DYRK1A gene.

P0784. Current models of mutagenesis applied to missense single-nucleotide substitutions in the human lamin A/C gene

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Autosomal dominant Emery-Dreifuss muscular dystrophy is caused by mutations in the gene, coding the lamin A/C nuclear lamina proteins. Surprisingly, mutations in the nuclear envelope proteins lead to muscular dystrophy. The pathological mutations in that case are mainly missense, which are most informative for understanding mechanisms of mutagenesis.
We analysed 27 missense substitutions of the lamin A/C gene, regarding their possible origin. Transitions account for 59.3% versus transversions 40.7%. Nine transitions (33.3%) in CpG islands could be explained by methylation-mediated deamination. We analysed the mutability at dinucleotide level. As expected the CG dinucleotide was most frequently affected. Single-base mutability was assessed on non-CpG mutations and the obtained order was: G=C>T>A.
Other mechanisms of mutagenesis (e.g. slipped-mispairing during replication) may require specific flanking sequences (arrest sites for polymerase a, direct repeats, palindromes and symmetric elements). We analysed 15 nucleotides upstream and downstream the mutation. Slipped-mispairing hypothesis is applicable in 37% of the cases. The arrest site for polymerase a was associated with 25.9% of mutations. Direct repeats, palindromes and/or symmetric elements were almost invariably present in all the analysed areas.
In conclusion, there is no a single mechanism, which can explain all the cases. Among the investigated mechanisms, the “environment”, i.e. the presence of repeated motifs seems to play a major role.

P0785. Outcome of three donor splice site mutations accounting for congenital afibrinogenemia and order of intron removal in the fibrinogen alpha gene (FGA).

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Congenital afibrinogenemia (MIM # 202400) is a rare, autosomal recessive disorder characterised by the complete absence of circulating fibrinogen. Our studies on the molecular basis of the disease showed that the most common genetic defect is a donor splice mutation in FGA intron 4, IVS4+1G>T. Two other FGA donor splice mutations, in intron 1 (IVS1+3A>G) and in intron 3 (IVS3+1_+4delGTAA) were identified in afibrinogenemia patients. Because it was impossible to directly study the effect of these mutations on mRNA splicing in patient hepatocytes we designed a transfected cell approach. For the common IVS4 mutation, multiple cryptic donor splice sites in exon 4 and intron 4 were found to be utilised. One of these, situated 4 bp downstream of the normal site was used in 85% of transcripts resulting in a 4 bp insertion-frameshift leading to premature truncation of FGA. Analysis of the IVS1+3 mutation showed intron 1 inclusion in the majority of transcripts, while the IVS3delGTAA mutation caused exon 3 skipping. The different outcomes of these donor splice mutations appear to follow the model proposed by Byers et al.(EJHG 2001, vol 9 suppl 1 p.80) in a study of fibrillar collagen genes, where donor splice mutations occurring in a rapidly-spliced intron with respect to upstream introns lead preferentially to exon skipping, while mutations in later-spliced introns lead to intron inclusion or cryptic splice site utilisation. Indeed, we found that in FGA introns 2 and 3 were spliced first, followed by intron 4 and finally intron 1.

P0786. DQA1 polimorphism analysis in Congenital Adrenal Hyperplasia (CAH) patients from Russia.

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Virilizing congenital adrenal hyperplasia (CAH) is the most common cause of genital ambiguity. 90-95% of CAH cases are caused by 21-hydroxylase deficiency. Particular forms of 21-hydroxylase deficiency are associated with particular combinations of HLA-antigens or haplotypes. Typing of DQA1 alleles (0101:0102, 0103, 0201:0601, 0301, 0401:0501) was carried out in DNA samples of 86 CAH patients (23 with salt wasting (SW) form , 13 with simple virilizing form (SV) , 50 with nonclassic (NC) form and of 50 unrelated healthy donors. Significant decrease of DQA1 0401:0501 alleles was registered in SW patients compared to SV , NC patients and controls (15%, 25%, 40% and 36% respectively; $p<0.05$). About 39% of the chromosomes in SW patients and 16% in SV patients had either major deletion or large conversions modifying the CYP21B gene. These mutations were predominantly identified in chromosomes with DQA1 0101:0102/0201:0601 alleles. Another major mutation 656A -G was registered in 22% of SW and 16% of SV groups. This nucleotide change corresponds to DQA1 0103/0401:0501 alleles. The frequency of DQA1 alleles in NC group of CAH patients is not different from this one in the control group. DQA1 alleles typing may be used, as additional method in prenatal diagnosis of CAH.

P0787. Mapping of a Candidate Region for Autism on Chromosome 2q32.

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Autism is a relatively common primary developmental disorder, with a significant genetic component. Routine investigations for the cause of autism, including chromosome and fragile X analysis, almost always are normal. There is a polygenic component to autism, and large sib-pair studies have been carried out by a number of groups worldwide. Several groups have identified associations between autism and a wide area of chromosome 2q, flanked by markers D2S364 and D2S2188 (Buxbaum et al & the IMGSAC respectively) which shows the strongest evidence for linkage. These results strongly suggest a predisposing gene(s) to autism within the 2q region.
We have recently identified a patient with high-functioning autism, who has a small but cytogenetically visible de novo deletion of chromosome 2q32 which falls within these linkage findings. This would appear to be the smallest known deletion of this part of chromosome 2, suggesting that the deletion includes a predisposing gene(s) for autism.
Fine mapping of the deleted region was carried out using markers from 2q32, to map the exact size of the deleted region. This reduces the very large linkage region by >75% to approximately 8.7 megabases. The region contains approximately 16 known genes and 16 ESTs, a number of which are potential candidates for autism.
We present results of a wider study for the presence of submicroscopic microdeletions, and of candidate genes, incorporating both linkage disequilibrium and mutational screening approaches in a panel of 77 Irish families with autism within the mapped region.
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P0788. Mutations of the PKD2 gene in families with autosomal dominant polycystic kidney disease in Czech Republic

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease.The disease is caused by mutations of the PKD1 and PKD2 genes. DNA presymptomatic diagnosis is performed in our referential laboratory, using highly polymorphic microsatellite markers for DNA linkage analysis of both genes. Presymptomatic DNA diagnosis was performed in 186 unrelated ADPKD families. Detection of PKD2 mutations was established in 39 families (in 9 families the disease was clearly linked to PKD2 gene and in 30 families with mild clinical course was not possible to exclude the linkage to PKD1 gene) and in 27 patients with end stage renal failure later than in 63 years.An affected member from each family was analyzed by heteroduplex analysis (HA). Samples which exhibited shifted bands on HA were amplified and after purification sequenced in both directions. Twelve mutations were identified, nine mutations unique for Czech population and four mutations from unique mutations were not presented. Four new unique mutations are the following: 1.nonsense mutation in

exon 1, 145 C>T (Q49X), 2.missense mutation in exon 4, 917 G>A (R306Q), 3.frameshift mutation in exon 4, 1078-1081 del C, 4.missense mutation in exon 5, 1258 A>G (R420G).Segregation of the mutation with the disease in each family was tested by HA or sequencing. Establishment of localization and type of mutations and genotype/phenotype correlation in affected families will improve presymptomatic DNA diagnosis and could help to assess the clinical prognosis of ADPKD patients.
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P0789. Type VII collagen gene (COL7A1) mutations survey in Italian patients affected by epidermolysis bullosa dystrophica

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Dystrophic epidermolysis bullosa (DEB) is a rare skin disorder showing clinical heterogeneity and transmitted either in dominant (DDEB) or recessive (RDEB) mode. All variants of DEB have been associated to mutations in type VII collagen gene (COL7A1). More than 200 mutations, often specific to individual families, have been disclosed in this gene and some of them have been shown to be characteristic of certain ethnic populations.
We report the survey of COL7A1 mutations in Italian DEB patients. From the analysis of 51 DEB families 42 mutations were identified, 18 of which are novel. Genotype-phenotype correlations were in line with the general rules already drawn in DEB. In the characterised families, we performed 70 analyses of the carriers of mutations and 3 prenatal diagnoses.
Six frequent mutations were identified in Italian RDEB patients, i.e. the 497insA, the 4783-1G→A, the 7344G→A, the 425A→G, the G1664A, and the 8441-14del 21. While the 7344G→A and the 425A→G mutations have been found in different populations, the remaining have been identified prevalently or only in Italian patients. The haplotype analysis, together with the common geographic origin of the patients carrying the frequent Italian COL7A1 mutations, has evidenced an ancestral common origin of the mutated alleles. Altogether the 6 frequent mutations cover about 43% of RDEB alleles in Italian patients and therefore they should be screened firstly in the patients not yet characterised at molecular level.

P0790. Albright's Hereditary Osteodystrophy: Screening for GNAS1 mutations by DHPLC and expansion of the mutation and polymorphism spectrum.

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Albright's Hereditary Osteodystrophy (AHO) results from heterozygous deactivating mutations in the GNAS1 gene and is associated with short stature, obesity, brachymetaphalangia, ectopic ossifications and learning disability. The inheritance is autosomal dominant but modified by genomic imprinting. Additional endocrine abnormalities, known as pseudohypoparathyroidism (PHP1a), are associated with mutations of the maternal but not the paternally derived GNAS1 allele. The AHO phenotype is heterogeneous and the diagnosis is difficult to confirm in the absence of PHPIa without access to GNAS1 mutation screening.
GNAS1 lies within a complex imprinting cluster at chromosome 20q13. It comprises 13 exons spanning 20kb and encodes the 394 amino-acid alpha subunit of the adenylyl cyclase stimulatory protein, Gs.
We have screened over 80 patients with features of AHO for GNAS1 mutations using DHPLC for exons 2-13, and sequencing for exon 1. To date we have identified fourteen mutations spread throughout the gene and two novel exonic variants, V36V and S54G which are likely to be polymorphic. In addition, we have identified a small GCC expansion in the 5'UTR of an affected patient, a common intronic deletion polymorphism close to a splice site, and five further intronic variants. These findings together with evidence for and against pathogenicity will be presented in more detail.

P0791. Easy detection methods for recurrent GJB2 mutations in the Greek population

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Mutations in the GJB2 gene encoding the gap-junction protein connexin-26 (Cx26) on chromosome 13q11 have been shown as a major contributor to prelingual, non-syndromic recessive deafness. A variety of techniques has been developed for screening the GJB2 gene for known and unknown mutations, especially the most frequent mutation in the Caucasian population, the 35delG. However, as there are almost a hundred sequence alterations identified so far, and their geographic distribution differs a lot, there is great interest for a rapid and easy method of identifying other mutations. Here we present easy screening techniques for the 3 recurrent GJB2 mutations in the Greek population, besides the 35delG. These mutations, K224Q, W24X and L90P, were originally identified by DGGE and/or direct genomic sequencing of the coding region. We developed ARMS-PCR for detecting the L90P and K224Q mutations and PCR-RFLP for the W24X mutation, using 6 previously genotyped samples. Additionally, 25 unrelated Greek patients (21 familial and 4 sporadic cases) with non-syndromic hearing impairment (NSHI), previously screened for the 35delG mutation and found to be negative (20 familial cases) or heterozygotes (1 familial and 4 sporadic cases), were screened for the 3 mutations. We found the L90P mutation in compound heterozygosity with the 35delG in a sporadic case using the newly standardized method.
The fast and easy detection of recurrent mutations can significantly contribute to the diagnosis of deafness, carrier detection and genetic counseling.

P0792. Intragenic deletion of the STS gene involving exon 9 in X-linked ichtyosis

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X-linked ichthyosis (XLI) is an inherited disease characterized by dark, adhesive and regular skin scales present at birth or early after birth. The primary defect of XLI is the deficiency of steroid sulfatase enzyme (STS), which hydrolyzes 3-beta-hydroxysteroid sulfates. The STS gene locus is located on Xp22.3. Reports in the literature indicate a complete deletion of STS gene and flanking sequences in 85-90% of XLI patients, while only 8 intragenic deletions and 11 point mutations have been reported. This study reports a Mexican patient with XLI and a novel partial deletion of the STS gene. XLI diagnosis was confirmed through STS assay in leukocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate. The STS gene was analyzed by PCR. STS activity was undetectable in the XLI patient (0.0 pmol/mg protein/h) and very low in his mother (0.32 pmol/mg protein/h vs 0.84 pmol/mg protein/h of normal control). PCR analysis showed no amplification of exon 9 and normal amplification of exons 1-8 and 10 of the STS gene. We have analyzed more than 120 XLI patients and this is the first deletion in exon 9 of the STS gene reported in the literature.

P0793. Novel mutations in two Mexican families with Norrie

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Norrie disease (ND) is an uncommon X-linked recessive neurodevelopmental disorder characterized by bilateral congenital blindness, one half of the cases present mental retardation and one third have sensosorineural deafness.
The ND gene is localized in Xp11.3 and has three exons, one untranslated and it encodes a polypeptide of 131 a.a. called norrina,

that has an important function in vascularization and differentiation of the inner retina.
More than 70 nonsense and missense mutations, a translocation, an inversion, and several deletions in the spectrum of ND have been reported. The high number of pathological changes in this small gene suggests that norrina conformation is extremelly prone to disruption from changes ocurring anywhere in its structure.
We describe 2 novel mutations in the ND gene in two mexican families. The affected individuals, showed typical ocular features of ND with deafness and mental retardation. Exons 2-3 of the Norrie gene were analyzed through PCR and DNA sequencing. Family 1, showed a missense mutation (A97P) within exon 3 in the patien and heterozygocity in the mother and sister. Family 2, present a deletion of 246 pb within exon 3 observed only in the patient.

P0794. Clinical and molecular characterisation of Osteogenesis imperfecta in patients from Lithuania

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Osteogenesis imperfecta (OI) is a heritable connective tissue disorder caused in >90% cases by dominant mutations in the genes COL1A1 and COL1A2, which encode the proα1(I) and proα2(I) chains of type I procollagen respectively. The severity of the OI phenotype is strongly associated with the polypeptide chain type, mutation site, flanking sequences, and residue substituted for glycine. The aim of the study was to evaluate clinical manifestation of OI on the molecular basis of disease.
Probands from 16 OI families (type I) and 22 sporadic patients with OI type I (11), type II (3) and type III (8) were screened for mutations in 40 exons of the COL1A1 gene using DNA heteroduplex analysis. Direct DNA sequencing revealed 10 OI (type I, III) causing mutations in 12 unrelated patients. Out of them, 8 (E500X, R183X, c.2165-2166insCTCTCTAG, c.1787delT, c.1786-1787insC, IVS19+1G>A, IVS20-2A>G, IVS22-1G>T) were null mutations (due to a premature stop codon arising either directly from a point mutation or indirectly from a frameshift mutation, or from a mutation causing an abnormality in mRNA splicing) leading to mild OI phenotype. OI was differently manifested in related patients with identical genotype and ranged from mild to severe phenotype in two families. This finding suggests that the phenotypic expression of the disease may be influenced by other factors (genetic or epigenetic), which may be important in the process of bone formation. The relation between location and nature of the glycine substitution and severity of OI was observed for expressed point mutations (G79R, G481A).

P0795. A new mutation in ferroportin 1 gene causes dominant inherited hemochromatosis

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Ferroportin 1 (FP1) is a human iron exporter expressed in enterocytes, macrophages, hepatocytes and placental syncytiotrophoblasts. FP1 coding gene, SCL11A3, consists of 8 exons and maps to 2q32.
Heterozygous mutations of FP1 cause hemochromatosis type 4 (HFE4). At variance with classic hemochromatosis HFE4 shows dominant inheritance, high serum ferritin before increased transferrin saturation and iron accumulation both in hepatocytes and macrophages. Two mutations of FP1 have been reported: (N144H) in exon 5 and (A77D) in exon 3.
We report the finding of a new FP1 mutation in 2 female patients (mother and daughter) with high serum ferritin, normal transferrrin saturation and signs of hepatic iron overload at liver biopsy and SQUID. Direct sequencing of the 8 exons of FP1 gene in both patients identified an heterozygous GTT deletion in exon 5, corresponding to a valine deletion in a valine triplet at position 160-162 (V162del) in the protein. The same mutation was not present in 50 normal controls.
The deletion occurs close to N144H in the highly conserved putative transmembrane domain involved in iron binding/transport. The finding of two different mutations in this domain provides further evidence of its relevance for FP1 function and suggests a loss of function as the mechanism of the disease. Our results add further support to the heterogeneity of hemochromatosis in Italy.

P0796. Molecular analysis of the TBX5 gene in patients with Holt-Oram syndrome

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Holt-Oram syndrome (OMIM #142900, McKusick 1986, syn: Heart-Hand syndrome) is a rare disorder involving developmental defects of heart and upper limbs. Main symptoms include ASD, VSD and defects of the thumb and radius. Malformations of the lower limbs never were described. The syndrome follows an autosomal dominant pattern of inheritance with complete penetrance and variable expression. This developmental disorder is associated with mutations in theTBX5 gene which plays an important role in the morphogenesis of heart and limbs in vertebrates. The TBX5 gene contains a highly conserved T-box DNA binding domain. The Holt-Oram phenotype results from haploinsufficiency of TBX5.
We performed the mutation analysis of the TBX5 gene by direct automated sequencing of the coding region (exons 2 to 9) and the exon-intron boundaries in 20 unrelated patients with various malformations of the upper limbs and heart, clinically diagnosed as Holt-Oram syndrome. In three cases a familiarity of the heart-hand defects occured. This analysis of the TBX5 gene identified four different mutations in five unrelated families. Three mutations were identified for the first time, only one mutation was described previously. All patients with disease related mutations in the TBX5 gene presented variable defects of the heart and upper limbs. Further studies involving clinical and genetic investigations are necessary to correlate specific mutations in the TBX5 gene with phenotype expression of the heart and hand defects.

P0797. Paternal 11p15.1-pter heterodisomy associated with the Beckwith-Wiedemann phenotype resulting from malsegregation of criptic familial translocation

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Beckwith-Wiedemann (MIM#130650) is a developmental disorder with variable phenotype and genetic heterogeneity. Pre and postnatal overgrowth, macroglossia, and anterior abdominal wall defects represent the main clinical signs of the syndrome associated with an increased susceptibility (7.5%) to a variety of embryogenetic tumors such as Wilms' tumors, adrenocortical carcinoma and hepatoblastoma. The BWS locus is subjected to genomic imprinting, with lesions involving a cluster of genes on 11p15.5. A small fraction (2%) of patients carry chromosome 11p15.5 abnormalities, duplications of paternal origin and balanced translocations or inversion with breakpoints on the maternal chromosome. Paternal uniparental disomy (pUPD) (20%), point mutations in the p57 gene (5-20%) and alteration in the methylation pattern of H19 and IGF2 imprinted genes are the most common pathogenetic mechanisms.
We refer on a patient displaying features such as gigantism, macroglossia, anterior abdominal wall defects and nephromegaly, consistent with BWS. The family records signaled two spontaneous abortions and a brother presenting with BWS features and nephroblastoma who deceased at four weeks. Standard karyotyping did not evidence chromosomal abnormalities. Microsatellite segregation analysis carried on to assess 11pUPD revealed paternal heterodisomy within 11p15.4-pter. By using the multiprobe telomeric FISH method the suspicion of a criptic translocation involving 11p15 was confirmed by the finding of an unbalanced translocation with derivative 21/11 in the proband and a balanced t(11;21)(11p15.4;21q 22.3) in proband's normal brother and father. Microsatellite analysis of 21q22.3-qter region allowed to map the 21q monosomy between D21S890 and qter. The contribution of 21q monosomy to the proband phenotype is under study.

P0798. ALK1 gene and Hereditary Hemorrhagic Telangiectasia (HHT): preliminary results of a screeening on 52 Italian families identify 13 new mutations.

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Hereditary Hemorrhagic Telangiectasia (HHT) (OMIM 187300) is an autosomal dominant disorder caused by mutations in either one of two genes: Endoglin (ENG, OMIM #131195) (HHT1) and Activin Like Kinase1 (ALK1, OMIM #601284) (HHT2). Evidence for a third locus has also been reported.
The clinical presentation typically includes epistaxis and telangiectasies; a diagnosis can be held for confirmed if 3 of the 4 suggested diagnostic criteria (epistaxes, telangiectasies, visceral lesions, positive family history) are present. The phenotype is highly variable and penetrance is complete by the age of 40 years. Arterovenous fistulae are frequently observed in liver, lungs and brain and may cause severe life threatening complication.
The number of mutations identified so far is limited; in particular for ALK1 only 24 mutations have been reported, studied in single patients or in very small samples. Here we report the preliminary results of the first screening conducted on 52 Italian families in which at least one subject results clinically affected by HHT. We analysed exons 3, 7 and 8 of the ALK1 gene by either SSCP or dHPLC techniques. We were able to identify 16 mutations, 13 of them are previously unrepoeted; 2 of them were found in 2 unrelated families with the same geographic origin. 6 mutations have been found in exon 3, which codifies for the extracellular receptor domain; 4 mutations in exon 7 and 4 in exon 8 which codify for the intracellular Tyrosin-kinase domain. Six of these patients (37,5%) present liver involvement.

P0799. Novel SCN5A Mutation Leading Either to Isolated Cardiac Conduction Defect or Brugada Syndrome in a Large Family

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The *SCN5A* gene encoding the human cardiac sodium channel α subunit plays a major role in cardiac electrophysiology. Mutations in *SCN5A* lead to a large spectrum of phenotypes including the long-QT syndrome, the Brugada syndrome and isolated progressive cardiac conduction defect (ICCD or Lenègre disease). In the present study, we report the identification of a novel *SCN5A* missense mutation causing either Brugada syndrome or ICCD in the same pedigree. In a large French family, we identified a G-to-A mutation at position 4372 that was predicted to change a glycine for an arginine (G1408R) between the DIII-S5 and DIII-S6 domains of the sodium channel protein. Among 45 family members, 13 were carrying the G1408R mutation. Four patients from 2 family collateral branches showed typical Brugada phenotype including ST segment elevation in the right precordial leads and right bundle branch block. One symptomatic patient with Brugada phenotype required implantation of a cardioverter-defibrillator. Seven patients from 3 other family collateral branches had ICCD but no Brugada phenotype (negative flecainide test). One patient with ICCD had episodes of syncope and required a pacemaker implantation. Expression study of the G1408R mutated SCN5A showed no detectable Na^+ current but a normal protein trafficking. We conclude that the same mutation in the *SCN5A* gene can lead either to Brugada syndrome or to ICCD. Our findings suggest that the consequence of the same *SCN5A* mutation may be individual or branch specific. Cosegregation of a modifier gene could explain these branch-specific phenotypic differences.

P0800. Mitochondrial mutations in non syndromic sensorineural hearing impairement : A large spectrum of mutations.

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Mitochondrial (mt) DNA mutations identified in maternally inherited hearing loss (MIHL) suggest an important role for mitochondria in the function of the inner ear. Fifty five families with non syndromic MIHL, eight sporadic patients with non syndromic hearing loss (NSHL) after aminoglycoside treatments and 29 sporadic patients with NSHL have been collected. For each proband the deafness was documented : age of onset, audiometry, temporal bones CT scan. ARNr 12S, ARNt leucine and ARNt serine were analysed by denaturing gradient gel electrophoresis and sequencing. The A1555G mutation was present and homoplasmic in a large family (19 affected patients).The affected subjects presented a congenital bilateral and sensorineural hearing loss. The deafness was severe to profound and age-stable. T7511C mutation was observed homoplasmic or heteroplasmic in two large french families.The age at onset of deafness was variable. The bilateral and sensorineural hearing loss was stable or progessive. The A3243G mutation, usually observed in syndromic deafness (MELAS and MIDD), was founded in one family with an isolated sensorineural hearing loss (4 affected patients). The hearing impairement was postlingual and evolutive with variable severity (mild to severe). No diabetes mellitus, cardiomyopathy or neurologic symptoms were associated. No mt mutation was observed in sporadic cases. In conclusion, we have observed a mitochondrial mutation in 7.2% of non syndromic MIHL patients with a low prevalence of A1555G (1.8%). A3243G was observed for the first time in a family with NSHL. These results suggest that many different mitochondrial mutations could be involved in non syndromic MIHL.

P0801. Brain Asymmetry In Beckwith-wiedemann Syndrome, A Marker For Paternal Isodisomy ?

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Hemihypertrophy is acommon finding of the Beckwith-Wiedemann syndrome (BWS) particularly in limbs and occasionally in the face or jaw. Brain hemihypertrophy is not commonly reported.
We describe a female patient with right hemihypertrophy noted at birth by right facial hemihypertrophy and jaw asymmetry. Limb involvement was also noted in lower more than in upper limbs. Right brain hemihypertrophy was found using visual evoked potentials (VEP) and persists after 14 years of follow-up.
Paternal isodisomy was demonstrated using molecular markers for the BWS critical region as published in 1993*. This patient has developed normally albeit with recurrent ear infections , right leg hypertrophy requiring 1cm sole on left leg and slight scoliosis. She has otherwise normal growth and development.
The question raised is whether this brain asymmetry reflected by the asymmetric VEP is simply a marker for the BWS or a marker for the isodisomy. The evidence on this issue will be discussed and recommendations made to routinely study the VEP in BWS patients.
*Henry I, Puech A, Riesewijk A, Ahnine L, Mannens M, Beldjord C, Bitoun P, Tournade MF, Landrieu P, Junien C. Somatic mosaicism for partial paternal isodisomy in Wiedemann-Beckwith syndrome: a post-fertilization event. Eur J Hum Genet. 1993;1(1):19-29.

P0802. Analysis of large structural changes of the factor VIII gene involving intron 22 in severe haemophilia A

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Haemophilia A (HA), the deficiency of the coagulation factor VIII (fVIII), is the most common severe, sex-linked inherited bleeding disorder. In approximately 50% of the patients with severe HA (fVIII level<1%), the disease is caused by fVIII gene intron 22 (IVS22) inversions, generally occuring as a result of an intrachromosomal recombination between the F8A gene located in IVS22 and one of its two extragenic homologous copies located telomeric from the fVIII gene. We studied 101 unrelated, severe HA-patients or obligate carriers by Southern blotting. We found known, altered inversion patterns in 54 (53%) cases: 38 (70%) distal, 12 (22%) proximal types and 4 (8%) unusual patterns. We observed loss or exclusively altered intronic bands in two cases of the latter group. We proved the existence of large deletions involving IVS22 and exon 22 or 23 by exon-specific amplification. The remaining two patients showed extra homologous F8A copies (4 bands in affected males) on Southern analysis that were not corresponding to type 3A or B inversions. To further analyse these two cases, “long-distance” PCR (LD-PCR) was

performed for separate amplifications of the intronic and extragenic copies of F8A. In both cases, LD-PCR showed a normal and an inversion-affected intronic copy of F8A and normal extragenic copies, suggesting that an extra (possibly inserted) intronic fragment participated in the inversion process. The present cases further support the theory that, the structure of fVIII IVS22 represents a hot spot for large gene rearrengements and emphasize the importance of alternative mutation mechanisms.

P0803. Results of mutation testing in >400 families affected by Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency

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21-hydroxylase deficiency is an autosomal recessive disease that presents with a range of symptoms. The late onset form is often so mild that it may be undiagnosed in affected individuals (cryptic CAH). Symptoms of classical CAH ranges from simple virilisation to a potentially life threatening inability to retain salt. There is a strong demand for prenatal diagnosis from couples who have already had an affected child. Prenatal diagnosis is usually carried out by linkage analysis with microsatellite markers closely flanking the functional 21-hydroxylase (CYP21) gene. Couples may opt for an in-utero treatment regime for affected female pregnancies.
The majority of the mutations causing 21-hydroxylase deficiency are known but testing for them is complicated by the complex arrangement of the genes responsible. We currently carry out testing for large scale deletions/conversions of the CYP21 gene by Southern blot analysis. We also screen for a common form of deletion and eight other mutations using direct ARMS tests. The CYP21 genes of affected individuals with one or more mutations undetected following these tests are sequenced in four overlapping fragments.
This strategy has identified both mutations in approximately 95% of >400 families tested and enabled us to assess the frequency of each mutation in British patients with 21-hydroxylase deficiency. In addition, this study has highlighted a number of interesting cases where for example there is evidence of mosaicism, *de novo* mutational events and parents with cryptic CAH. These observations indicate the need for mutation screening prior to prenatal diagnosis by linkage analysis wherever possible.

P0804. Further evidence for locus homogeneity in the Marfan syndrome (MFS)

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The clinical diagnosis of MFS, an autosomal dominant multisystemic disorder, is based on a set of major and minor criteria, known as the "Ghent nosology". MFS is caused by mutations in the fibrilline-1 gene (FBN1). More than 200 FBN1-mutations have been identified. However, due to incomplete mutation uptake and the suggestion of a second locus on chromosome 3p, the issue of locus heterogeneity in MFS remains under debate.
We present results on a cohort of 103 patients fulfilling the clinical diagnosis of MFS according to the Ghent nosology, in which a thorough mutationanalysis was done by CSGE/SSCP followed by DHPLC or sequencing. Initial mutationscreening allowed to identify an FBN1-mutation in 74 patients. Next, sequencing of all FBN1-exons was performed in 16 patients and screening by DHPLC in 13 patients, identifying respectively 7 and 5 additional mutations. In 5 more patients with a positive family history of MFS, but no mutation identified, segregation analysis showed linkage to the FBN1-locus. In 12 patients (~10%) the involvement of FBN1 could not be proven by this approach. The phenotype of these patients did not differ from the others with respect to the distribution of major clinical manifestations. Southern blot analysis to exclude large deletions in the FBN1-gene is in progress. Most likely, a portion of FBN1-mutations remains undetected because of technical limitations.
In conclusion, the involvement of the FBN1-gene could be demonstrated in ~90% of all MFS patients, which strongly suggests that this gene is the predominant if not the sole locus for MFS.

P0805. Identification Of A Novel Missense Mutation (q283p) In A C282y Heterozygote Hemochromatosis Proband From Brittany (western France)

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Introduction : Depending on the population studied, between 4 and 35% of hemochromatosis probands have a least on chromosome without an assigned HFE mutation (i.e. C282Y, H63D and S65C). In the present study, we have performed a D-HPLC scanning of the HFE coding region in twenty HC probands from Brittany to define if they carry uncommon mutations liable to explain their iron overload phenotype. Material : The 20 HC probands were selected because a transferrin saturation above the threshold (³ 60% in men ³ 50% in women) in absence of others known cause of iron overload. Results : D-HPLC scanning had allowed us to detect a novel missense mutation in exon 4 of the HFE gene from one proband who was also C282Y heterozygous. Subsequent sequencing analysis identified an adenine to cytosine transversion at position 848 which changes amino acid 283 from glutamine to proline (Q283). Another family member (sister) was also found C282Y/Q283P compound heterozygote , presenting with a transferrin saturation level of 74%. Theoritical structural analysis shows that folding of HFE is blocked by a proline in position 283 which cannot complete the H-bonding network and could have a deleterious effect on the key disulphide bridge in the a3 domain. Conclusion : With the description of ten other rare or private causative mutations, our study confirms that it may be of particular interest to perform a complete analysis of the HFE coding region in HC probands with at least one chromosome lacking the C282Y, H63D and S65C mutations.

P0806. Fabry disease: Exclusion of D313Y as a disease causing mutation of the alpha-Galactosidase A (GLA) gene.

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Fabry disease is a X-linked recessive metabolic disorder caused by mutations in the alpha-Galactosidase A (GLA) gene. The identification of the disease causing mutation in a given Fabry family permits precise genetic counselling, heterozygote detection of female family members and prenatal diagnosis.
We present a large pedigree of a German Fabry family with mutation analysis in more than thirteen individuals. All affected males had died. Mutation analysis in a putative female carrier (suspected by biochemical GLA measuring) showed the sequence variant D313Y of the GLA gene. D313Y was previously described as a disease causing mutation by Eng et al. in 1993. In a prenatal diagnosis (CVS) of a male pregnancy we detected the D313Y sequence variant. In order to confirm our results, we performed a biochemical GLA measurement. Unexpectedly the CVS cells showed an alpha-Galactosidase A activity in the normal range.
Molecular genetic investigations in further family members (i) identified the D313Y variant in a healthy 49 year old male and (ii) excluded this sequence variant in an obligate female carrier, whose DNA became meanwhile avaible for genetic analysis. Genomic DNA sequencing in this individual revealed the nonsense mutation W348X in exon 7 of the GLA gene. Pedigree analysis confirmed that W348X, as distinct from D313Y, cosegregates with the disease in the family. Our results show that the D313Y sequence variant of the GLA gene is an amino acid substitution, whose influence -if at all- on the severity of the Fabry phenotype remains to be clarified.

P0807. Spectrum of mutations found in 86 cases with Noonan syndrome (NS)

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Noonan-syndrome (NS, MIM 163950) is a well recognized autosomal dominant multiple malformation syndrome with an estimated incidence of 1 in 1,000 to 2,500 live births. Affected individuals have proportionate short stature and a characteristic facial appearance with hypertelorism, ptosis, downward slanting palpebral fissures, and low set posteriorly rotated ears. In addition, cardiac involvement, most commonly pulmonary valve stenosis and hypertrophic cardiomyopathy, is frequently seen. NS is genetically heterogenenous. Linkage to a 5 cM region on chromosome 12q24 has been reported previously and only recently, mutations in the protein tyrosine kinase gene PTPN11, have been described. In a small sample, missense mutations were found in more than 50% of NS cases (Tartaglia, M et al., Nat. Genet. 29: 465-468, 2001). We have screened PTPN11 for mutations in 86 clinically well characterized familial and sporadic NS cases and identified 14 different missense mutations in 29 (34%). Seven mutations, 5 in the N-SH2 domain, 1 in the C-SH2 domain and 1 in the PTP domain, are novel and have not been described before. In addition, we found 4 of 7 mutations described by Tartaglia et al (Asp61Gly, Tyr63Cys, Ala72Ser, Asn308Asp) repeatedly in apparently unrelated cases. Most mutations cluster in the SH2 domain at the N-terminus (N-SH2), which acts as a molecular switch between the inactive and active protein form. No PTPN11 mutations were detected in 4 patients with cardio-facio-cutaneous syndrome (CFC), which shares many phenotypic similarities with NS.

P0808. Mutation screening of the BIGH3 gene in patients with Keratoconus.

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Keratoconus has an approximate incidence of 50-230/100,000 in the general population. Progressive non inflammatory corneal thinning is a characteristic feature of this disease. The age of onset is at puberty and the disorder is progressive until the third to fourth decade of life when it usually arrests. It is a major cause of cornea transplantation in developed countries. Genetic factors have been suggested as a cause of keratoconus. Both autosomal dominant and autosomal recessives forms of vertical transmission/inheritance have been suggested. There are many reports suggesting involvement of BIGH3. The BIGH3 gene is expressed in the cornea and localizes to human chromosome 5. Mutations in this gene are responsible for causing corneal dystrophies. In addition, the protein levels of BIGH3 are reported to be altered in keratoconus tissues. We screened 16 individuals, representing different families with keratoconus and for mutations within the BIGH3 gene. Although we found more than 6 sequence variations we did not find any protein altering changes. We concluded that the BIGH3 gene is not responsible for causing keratoconus in this patient population.

P0809. Investigation of LHON Primary Point mutations in Iranian Patients

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Abstract
PURPOSE: To define the prevalence of a panel of mitochondrial DNA (mtDNA) mutations associated with Leber's hereditary optic neuropathy (LHON) in the Iranian LHON population. LHON-associated mtDNA mutations have been found in LHON patients from around the world, but the Iranian LHON population has not been studied. METHODS: 23 Iranian patients were defined clinically as having LHON on the basis of painless, subacute, bilateral optic neuropathy and the exclusion of other causes of subacute optic neuropathy. MtDNA was extracted from blood of the probands and healthy members of their families and assayed for a panel of primary LHON-associated mtDNA mutations by polymerase chain reaction (PCR)-based methods. We studied four well-known LHON-associated primary mutations (at nucleotide positions 11778, 3460, 14484, and 14459) in all 23 probands. RESULTS: Among the 23 probands tested for four common LHON mutations, 3 carried the 11778 mutation, 1 carried the 14459 mutation, 1 carried the 3460 mutation and no one carried the 14484 mutation. The phenotype of female of members of family who carried 11778 mutation showed the normal variant even they had the same level of this mutation. Beside one patient who carried 3460 mutation all of our patients were male. We could not find any new point mutation in ND6 gene of other patient even this gene is hot spot for LHON. CONCLUSION: The results of mtDNA analysis of the Iranian LHON patients appear to be the same from those of previous reports.

P0810. Mutations in the GJB-2 gene in patients with non-syndromic hearing loss from endogamous population of Roms (Gypsies)

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Mutations in the connexin 26 gene (GJB-2) represent a major cause of autosomal recessive non-syndromic hearing loss (NSHL) worldwide. In most Caucasian populations, the 35delG mutation in this gene was found to account for up to half of the genetic non-syndromic childhood deafness. In populations of non-European ethnic background, other GJB-2 gene mutations are occasionally common, e.g. 167delT in Ashkenazi Jews, R143W in Africans, 235delC in Koreans.
DNA samples from 54 unrelated NSHL patients from endogamous and inbred population of Roms (Gypsies) from Eastern Slovakia were screened for two GJB-2 mutations: 35delG and W24X. A single patient was found homozygous for 35delG, 8 were homozygous for W24X, 5 compound heterozygotes 35delG/W24X, 3 heterozygous for W24X (and an unidentified mutation), and one heterozygous for 35delG (and an unidentified mutation). In remaining 36 patients, no GJB-2 mutation was found. Thus, in Slovak Roms, W24X accounts for 23,52 %, whereas 35delG for only 5,88 % of all GJB-2 gene mutations in NSHL patients. So far, the W24X mutation was observed in two Pakistani NSHL families, which is in accordance with the hypothesis of Indian origin of European Roms.

P0811. Analysis of polymorphism for UGT1*1 exon 1 promotor in neonates with pathologic and prolonged jaundice

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The role of Gilbert's syndrome, which is associated with unconjugated hyperbilirubinemia and decreased bilirubin UDP-glucuronosyltransferase activity, in neonatal jaundice is still not well known. We aimed to investigate whether a TATA box polymorphism in the promoter of the UGT1*1 exon I, the most common detected DNA polymorphism in Gilbert's syndrome, is a contributory factor in unexplained pathologic or prolonged jaundice. 38 neonates who had unexplained pathologic jaundice, 37 neonates who had unexplained prolonged jaundice, and 35 healthy, nonjaundiced neonates were enrolled in the study. Genomic DNA was isolated from blood and polymerase chain reaction amplification was used to examine sequence variation of the promoter upstream of UGT1*1 exon I. Genotypes were assigned as: 6/6 (homozygous for a normal allele bearing the sequence (TA)6TAA), 7/7 (homozygous for an abnormal allele with the sequence (TA)7TAA), and 6/7 (heterozygous with one of each allele). Of the 110 infants, 10 (9%) had 7/7, 51 (46%) had 6/7, and 49 (45%) had 6/6 genotype. Although the percentage of 7/7 was higher in the group with pathologic jaundice (13%) than in the group with prolonged jaundice (8%) and in the control group (6%), the difference between three groups were not statistically significant. Also no differences were observed among different genotypes and mean serum total bilirubin concentrations. In conclusion, we showed that TA 7/7 and TA 6/7 genotypes are not rare in our population and the

presence of those polymorphisms alone does not play a significant role in the etiology of unexplained pathologic or prolonged neonatal hyperbilirubinemia

P0812. An efficient strategy for molecular diagnosis of Wilson disease in the Sardinian population.

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Wilson disease (WD) is an autosomal recessive disorder of copper transport resulting from mutations in the ATP7B gene. Mutation analysis of the ATP7B gene carried out in 76 unrelated WD Sardinian families allowed the characterization of 94% of the chromosomes analyzed and led to the identification of 16 WD causing-mutations (Hum Mut .14: 294-303, 1999). Six of the mutations identified are frequent and account for the molecular defect 85% of WD chromosomes. In an effort to increase our capability of molecular diagnosis of WD in Sardinians we have set up a multiplex PCR method for the detection of the 6 most frequent mutations, coupled with reverse dot blot analysis. Primers and ASO probes sequences were designed to permit common experimental conditions during the multiplex PCR and reverse dot blot analysis respectively.
Using this procedure, we confirmed the results obtained in the patients previously characterized by SSCP analysis. Molecular diagnosis of an additional group of 51 DNA samples of Sardinian origin revealed again concordant results using both reverse dot blot and SSCP methods. On the basis of these data we conclude that the most efficient strategy for molecular diagnosis of WD in Sardinian population consists in a preliminary mutation screening for the six most common mutations using multiplex PCR followed by reverse dot blot analysis. Samples not characterized by this first step of analysis will be subsequently analyzed using SSCP analysis for all the exons and the promoter region of ATP7B gene.

P0813. Holoprosencephaly : functional analysis of human SHH missense variants

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Holoprosencephaly (HPE ; 1,2/10.000 live births ; 1/250 conceptuses) is a common development defect affecting both the forebrain and the face. Clinical expressivity is variable, ranging from a single cerebral ventricule and cyclopia to clinically unaffected obligated carriers in familial HPE. The disease is genetically heterogeneous but additional environmental agents also contribute to the aetiology of HPE. This study includes 126 unrelated nonchromosomal HPE cases (76 typical HPE, 25 atypical cases, 25 polymalformative cases). We provide clinical data regarding the subgroup of typical HPE and report 21 novel heterozygous mutations (16% for all the cases, 25% for typical HPE), 12 in Sonic hedgehog gene (SHH), 5 in ZIC2, 3 in SIX3, and 1 in TGIF. Ten mutations were found in familial cases whereas 11 mutations were identified in apparently sporadic cases. In addition to clear loss-of-function mutations conferred by nonsense or frameshift alteration in the coding sequence, genetic screening has revealed missense codons with less obvious functional consequences. The ability to discriminate between a loss-of-function mutation and a silent polymorphism is important for genetic testing for inherited diseases like HPE where the opportunity for prenatal diagnosis may be considered. We report here a functional test where the significance of SHH aminoacids replacements observed in the human population is tested by the C3H10T1/2 osteoblast transformation and phosphatase alcaline production under the Sonic Hedgehog action.

P0814. Mutations in the gene for SLURP-1 in patients with Mal de Meleda (MDM) with recessive and pseudo-dominant inheritance

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Mal de Meleda (MDM), or keratosis palmoplantaris transgrediens of Siemens, is a hereditary skin disorder characterised by diffuse palmoplantar keratoderma (PPK) and transgressive keratosis. There is no associated involvement of other organs, however, a rather broad spectrum of clinical presentations with other variable features is characteristic. MDM was first described in patients from the isle of Mljet (Meleda) in Croatia. Recently, mutations in the ARS (component B)-81/s gene on chromosome 8q24-qter were identified in patients with MDM. We have shown lately that a very similar phenotype of transgressive PPK is not linked to chromosome 8q24-qter in several families from the United Arab Emirates. Here we present further families with transgressive PPK. Three novel mutations in ARS (component B)-81/s were identified in consanguineous families from Turkey, Palestine, and the United Arab Emirates: two different mutations affecting the same codon, both resulting in the amino acid change G86R, and a mutation that alters the translation initiation codon. In a German family without known consanguinity, which was originally supposed to have a dominant form of transgressive PPK, we demonstrated a pseudo-dominant inheritance. Three children and their affected mother were homozygous for the mutation W15R while the unaffected father was heterozygous. Pseudo-dominance was confirmed by the analysis of several neighbouring microsatellites. Our findings show that the MDM type of transgressive PPK may be caused by SLURP-1 mutations in patients from various origins. A founder effect is supposed to be responsible for MDM on Mljet, however, here we demonstrate allelic heterogeneity for mutations in SLURP-1.

P0815. A frequency of Cx26 mutation 35delG in patients with hearing loss.

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Mutations in the gene for connexin 26 are the major cause of autosomal recessive inherited non-syndromic congenital hearing loss. A single mutation 35delG is responsible for the majority of affected alleles in different populations. In several Russian populations average carrier frequency of 35delG mutation is 1/46.7. We have analyzed 109 unrelated patients affected by sensoneural hearing loss for 35delG mutation. All of ours patient had early onset deafness with various degree of hearing loss. First group consisted 87 patients with normal hearing parents, in second group19 patients had both parents with hearing loss, in 3 patients only one parent had hearing loss (third group). In the first group we have found 33 families (38%) with mutation 35delG. In 22 patients mut 35delG was in homozygous state, in 11 patients it was in heterozygous state. In the second group all the patients had mut 35delG. Sixteen patients (84%) had mut 35delG in homozygous state; others had mut 35delG in heterozygous state. In the third group mut 35delG have been found in one patient in heterozygous state. We have accounted these results on a percent of chromosomes affected by mut 35delG. In the group of patient with normal hearing parents quota of injured chromosomes was 31.6%, in group of "deafness" families it was 92%. Summer percent of chromosomes with mut 35delG was 41.7.

P0816. Emilin Family Genes Are Not Involved In Marfan-like Phenotype

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Elastic fibers are major constituents of the extracellular matrix and confer to connective tissues the properties of resilience and elastic recoil. Recently EMILIN1 and EMILIN2, largely distributed in connective tissues, have been isolated. The structural components of elastic fibers have been found to be defective in some heritable human diseases, such as Williams syndrome and familial supravalvular aortic stenosis for elastin and Marfan's syndrome and congenital contractural aracnodactyly for fibrillin. . However, no mutations responsible for many diseases due to anomalies of elastic fibers/connective tissue have been detected. Since EMILIN1 and EMILIN2 are found only within elastic fibers, and they co-react, mutations of these gene family are expected to give rise to alterations of this extracellular matrix component. We have searched

for mutations of EMILIN1 and EMILIN2in 8 families with Marfan-like phenotype.
For each family a three-generation pedigree, physical examination of all probands and their first degree relatives has been performed. DNA was obtained after informed consent of probands and first degree relatives.
A mutation analysis has been performed using SSCP and CSGE techniques.
At present no mutations have been identified in the families. Although the low number does not allow us to make final conclusions, EMILIN family genes seem not to be involved in Marfan-like phenotypes.

P0817. Four new mutation and neutral polymorphisms of the low density lipoprotein receptor gene in St. Petersburg familial hypercholesterolemia.

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Familial hypercholesterolemia (FH) is an inherited metabolic disease with a dominant mode of inheritance. It is quite common in most human populations (1:500) and results in reduction of low-density lipoprotein (LDL) catabolism followed by premature coronary heart disease. The disease is caused by mutations in the LDL receptor gene.
In order to develop presymptomatic diagnostic tools for management of the FH we aimed to study the LDL receptor gene mutation spectrum in St.-Petersburg (Russia). We have created a DNA bank from 100 unrelated patients with clinical picture of FH. Earlier, seven mutations in the LDL receptor gene were found in some of the probands. In view of the growth of the collection of DNA samples, we continued the search for genetic abnormalities in exons 4 and 10 of this gene. This exons were amplified by polymerase chain reaction (PCR) and screened for presence of the mutations via combined single-strand conformation polymorphism -heteroduplex analysis (SSCP-HA). The fragments showing shifted mobility in polyacrylamide gel electrophoresis were sequenced by method of Sanger. Up-to-date, we have identified four new mutations - A130P, G128G, C146R, C188Y. Rapid methods for mutation detection were developed. Cosegregation of mutations and high cholesterol levels proves the role of mutations in disease development. Two polymorphic sites in exon 10 of the LDL receptor gene (1413G/A and 1545C/T) were found in the Russian population for the first time. Based on the data obtained, familial hypercholesterolemia was confirmed in seven patients.

P0818. First description of a recessive form of Central Core Disease, transitorily presenting as Multi-minicore Disease and associated with a homozygous mutation in *RYR1*.

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Multi-minicore Disease (MmD) is an autosomal recessive congenital myopathy characterized by the presence of multiple small zones of sarcomeric disorganization and mitochondria depletion ("minicores") in muscle fibers. Its clinical phenotype is heterogeneous. To identify the genetic basis of the disease, we performed a genome-wide screening in a consanguineous Algerian family in which 3 children presented in infancy with generalized muscle weakness, more evident in pelvic girdle and hands, and joint hyperlaxity ("distal involvement" phenotype). Their first muscle biopsies (at 2 and 4 years of age) showed multiple minicores. By homozygosity mapping, linkage of the disease to the 19q13 region was identified in this family and, subsequently, in 3 additional MmD families showing a similar phenotype (LS=5.19 for D19S570 at θ=0.00).
A gene encoding the skeletal muscle ryanodine receptor, *RYR1*, is located in this region. Heterozygous *RYR1* mutations cause 2 autosomal dominant entities: the congenital myopathy Central Core Disease (CCD) (C-terminal domain mutations) and Malignant Hyperthermia Susceptibility (N-terminal domain mutations). In the Algerian family, we identified the first homozygous missense mutation (P3527S) in the central part of *RYR1*. New muscle biopsies at adulthood revealed typical CCD with rods in the 3 patients and absence of cores in the healthy parents.
This subgroup of families linked to 19q13 constitute the first variant of CCD with genetically proven autosomal recessive inheritance and transient presentation as MmD. This work also illustrates the age-related modification of the morphological lesions in congenital myopathies, and represents the first description of a genetic defect underlying the MmD phenotype.

P0819. Mutation analysis in Crouzon and Pfeiffer syndromes identifies novel substitutions in the tyrosine kinase regions of the fibroblast growth factor receptor-2 (FGFR-2)

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Crouzon and Pfeiffer syndromes are autosomal dominant craniosynostoses characterised by premature fusion of one or more cranial sutures and dysmorphic facial features with or without limbs abnormalities. These conditions are associated with heterozygous mutations in three members of the fibroblast growth factor-receptor family (FGFR1-3) but the great majority of them are missense substitutions exclusively located in the extracellular domain of FGFR2. Studying a large series of 73 unrelated patients, we found 28 different heterozygous mutations in 66 cases (90%). This included 22 "conventional" mutations in 59 individuals (the P252R mutation in FGFR1, the A391E mutation in FGFR3, and 20 different mutations in exons IIIa and IIIc of FGFR2) and 6 novel FGFR2 substitutions in 7 patients. Interestingly, two of these (N549H and K641R) were found in the intracellular domain of FGFR2, within the two tyrosine kinase regions of the receptor and were associated with Crouzon syndrome (2 cases) and Pfeiffer syndrome (1 case), respectively. Sequence alignments of FGFR2 and FGFR3 showed that asparagin 549 in FGFR2 corresponds to asparagin 540 in FGFR3 which is mutated in hypochondroplasia (N540K) suggesting that the N549H substitution may be an activating mutation of FGFR2. Extensive screening of intracellular domains of FGFR1 and FGFR2 are now being investigated for the 10% remaining cases of our series. These results show that FGFR2 mutational spectrum is not restricted to the Ig-loops of the extracellular region as previously thought and suggest that all Crouzon and Pfeiffer cases are associated with FGFRs mutations.

P0820. Molecular analysis of the RET proto-oncogene on MEN II patients

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Multiple endocrine neoplasia type II syndromes (MEN IIA and IIB) are an autosomal, dominantly inherited disorders characterized by the occurrence of medullary carcinoma of the thyroid (MTC), hyperparathyroidism and pheochromocytoma. These syndromes result from mutations of the RET proto-oncogene on chromosome 10. Germ line mutations are also seen in patients with familial medullary carcinoma of the thyroid (FMTC). MTC is associated with high morbidity and mortality. The use of direct DNA testing is an attempt to improve the cure rate because in more than 90% of individuals with a RET proto-oncogene mutation, MTC will develop.
The aim was detection of RET protooncogene molecular anomalies causing MEN II syndromes. We have studied 5 familial and 7 sporadic cases of the MEN IIA syndrome and 19 sporadic MTC cases. In all of these familial, 5 sporadic cases of MEN II A and 6 MTC cases direct sequence and restriction analysis of the exon 11 revealed the most common cysteine-to-arginine change in the codon 634 (TGC→CGC). Results of SSCP and heteroduplex analysis of the 10 and 11 exons in 2 remaining patients with sporadic MEN II A and 13 individuals with sporadic MTC were negative, but some of them have shown mobility shifts at 12-14 exons. Anomalies are characterized. In 2 families with MEN IIB syndrome a common germ line mutation of codon 918 was detected.

Early diagnosis by direct DNA testing led to early treatment with curative total thyroidectomy in two children from MEN IIA families and make possible prenatal diagnostics.

P0821. Molecular characterisation of X-linked properdin deficiency in a large French family

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Properdin deficiency type I, is a rare X-linked disorder (MIM 312060) strongly predisposing to meningococcal disease. Properdin stabilises the labile C3 convertase of the alternative pathway of the complement system. We report a large French family with four properdin deficient males in three generations: an affected grand-father with four daughters and three diseased grand-sons. All but one developed meningitis caused by *Neisseria meningitidis* serogroup Y or serogroup B. No functional activity of properdin was detected in the affected males. The properdin level was bellow the detection limit in two of them and deeply decreased in the others. In three obligate carriers, the properdin level was reduced near half of the standard value (14, 15, 17 mg/l; controls = 22-38 mg/l). One expressed a normal rate (25,3 mg/l). By sequencing long-PCR products of the properdin gene (*PFC* gene) we found a base change at the 3'end of the consensus sequence of intron 8 (namely 1205-1G>T). This mutation inactivates a splice site in the *PCF* gene as demonstrated by analysis of the leukocytes transcription products in affected patients. It is to date the first splice mutation demonstrated in this gene. Interestingly, one among the four female carriers developed meningitis, and shared with her two carrier sisters, chronic rheumatic diseases associated to marker HLA-B27. HLA-B locus and properdin factor B (C3 proaccelerator) being closely linked on chromosome 6, relationship between properdin deficiency and chronic rheumatic diseases will be discussed.

P0822. A non-glycine mutation in the C-propeptide of the alpha1(I) collagen chain causes mild Osteogenesis imperfecta and EDS-like features

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Osteogenesis imperfecta (OI) is a heritable connective tissue disorder characterized by a wide range of mutations occurring in the genes encoding type I collagen (COL1A1-COL1A2). These mutations mainly represent single-base changes resulting in the substitution of critical glycine residues but also deletions and insertions are reported.
We studied a 10-year-old girl with mild type I OI. The proband's delivery was complicated by fracture of the clavicle and pneumothorax. Postnatally, no further fractures occurred. To date, the patient has blue sclera and suffers from mild hyperlaxity of skin and joints.
We analyzed type I collagen production at the protein and molecular level. Although no abnormalities were observed on protein analysis, we identified a de-novo c3890A>G transition within exon 49 of the COL1A1 gene, resulting in a M1264V substitution in the C-propeptide. This transition causes the activation of a cryptic splice site inside the exon. As a result, the last 25 bp of exon 49 are spliced out, and due to a frameshift, a premature stopcodon is created in exon 50. The normal and both aberrant cDNA transcripts (11/ 20 clones carry the 25-bp deletion, 2/20 clones M1264V substitution) are detected.
At the protein level, the frameshift mutation results in the removal of 5 of the 6 critical cysteine residues in the C-propeptide, necessary for the incorporation of the chain in collagen type I molecules. The mild phenotype of this patient suggests that this latter protein defect is predominant and causes haploinsufficiency. Further biochemical characterization will be performed.

P0823. A novel mutation in the SDHD gene in a family with inherited paragangliomas

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Paragangliomas (OMIM# 168000), (carotid body tumours) are vascularized tumors of the head and neck. They may be unilateral, although with inherited predisposition, they tend to be bilateral. It has been observed that paragangliomas are often inherited via the paternal line; thus maternal imprinting occurs. This helped in finding linkage to 11q23-qter (Heutink et al. 1992), which led to the identification of a mutated gene, SDHD, which encodes the small subunit of cytochrome b in the succinate-ubiquinone oxidoreductase complex (PGL1) (Baysal et al. 2000). This enzyme complex, constituted from SDHA, B, C and D, is important for the tricarboxylic acid cycle and the aerobic respiratory chains. Thus, loss of SDHD may lead to chronic hypoxic stimulation of cellular proliferation that leads to tumorous growth. Mutations in the SDHB and C genes (PGL3 and PGL4) have also been identified, but in families with non-imprinted inheritance of paragangliomas (Niemann, and Muller, 2000; Astuti et al., 2001). Interestingly, another PGL locus was mapped more telomerically on 11q (PGL2).
We report a family with three generations affected with paragangliomas. Generation skipping of phenotype was noted once, when the disorder seemed to be inherited from the mother. Thus, the SDHD gene became the most likely candidate. In fact, we identified a novel splice site mutation that co-segregated with the phenotype. Interestingly, six non-affected carriers were observed, all of which had inherited the mutant allele from their mother. The identification of carriers enables genetic counseling, an important aspect for these usually treatable vascular tumors. (vikkula@bchm.ucl.ac.be)

P0824. Molecular pathologies of genes in the Hedgehog signalling pathway

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"Hedgehogopathies" is a term which designates a group of hereditary dysmorphic syndromes where an alteration of the Hedgehog signalling pathway in vertebrates occurs. Many actors in this transmission pathway have been identified by genetic studies in *Drosophila* ; their homologues in man are candidate genes for dysmorphic syndromes.
In humans, *Sonic Hedgehog* is mutated in Holoprosencephaly, *Patched* in Gorlin syndrome, and *GLI-3*, the homologue of the *Cubitus interruptus* gene in *Drosophila*, is implicated in Greig syndrome and in Pallister-Hall syndrome. Its co-activator, the transcription factor *CBP* is mutated in Rubinstein-Taybi syndrome. Finally, one of the target genes of *SHH*, *Pitx2*, is responsible for Rieger syndrome.
We have implemented genetic diagnosis of Gorlin syndrome (OMIM 180500), of Greig (OMIM 175700) and Pallister-Hall syndromes and also of Rieger syndrome (OMIM 180500).This work allowed us to consider phenotype-genotype relationships of these different hereditary diseases. We identified 9 novel mutations of the *Patched* gene, 4 novel mutations of the *Gli3* gene and one new mutation of the *Pitx2* gene. While a correlation exists between the mutation spectrum and Greig and Pallister-Hall syndromes, no correlation is found in Gorlin syndrome. Finally, the Reiger syndrome is characterized by major genetic heterogeneity. Clinical data and molecular results are discussed in the light of our understanding and knowledge of the Hedgehog signaling pathway in vertebrates.

P0825. Clinical heterogeneity in a family with M34T variant in the GJB2 gene.

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Mutations in the GJB2 gene have been found in a great number of

familial and sporadic cases of congenital deafness. This gene is responsible for more than 20% of prelingual recessive hearing loss in Caucasian populations, mainly due to mutations 35delG, and 167delT. Furthermore, the involvement of GJB2 gene in autosomal dominant deafness is also proposed but remains uncertain. One of the more controversial allele variants, M34T, has been hypothesized to cause as well an autosomal recessive, as a dominant nonsyndromic hearing loss, and even a null allele in the GJB2 gene. Recently, studies extending the analysis of the genetic background in the allele with the M34T mutation have identified a 10bp deletion (-493del10) in the non-coding region upstream of exon 1. In this study, we present a family with three sibships compound heterozygotes M34T/V95M with different degrees of hearing impairment. The proband has a mild hearing loss. His sister and his brother have a moderate hearing loss but presenting different audiometric data. Moreover, his mother has normal hearing but his father who is heterozygous for M34T mutation has a mild hearing loss. The effect of M34T mutation remains to be deciphered, and certainly its interpretation becomes more difficult in terms of genetic counseling. We understand that presenting different clinical situations, the correlations of genotypes/phenotypes may lead to new prospects and comprehension of patophysiology of the mutation.

P0826. First report on the genetics of prosopagnosia

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Prosopagnosia (PA), a term introduced by Bodamer (1947, Arch Psych Nerven 179:6ff), defines the inability to associate a face with a certain person, while a face is still recognized as such. The specifity of this deficiency is supported by double dissociation between probands with impairment of e.g. object agnosia but not prosopagnosia and vice versa. Almost all reports (#259 citations in medline[R]) are single casuistics or collections of unrelated patients who acquired prosopagnosia after brain injuries, strokes, or atrophy of at least the right occipito-temporal area.
There are only a few reports of congenital PA – all of them sporadic cases (#4 citations in medline[R]). The only hint of familial recurrence is found in a report of McConachie (1976, Cortex 12:76ff) with PA in mother and daughter. There is no hint in this publication that the author examined the family tree in any more detail. No entry was added to V. McKusick´s database (MIM). Later, Farah et al. (2000, Cognit Neuropsychol 17:117ff) hypothesized that PA and other agnosias are "... explicitly specified in the genome".
We have found 3 distinct families of at least 5 persons each with congenital PA ascertained in 4 generations. This indicates a much higher prevalence than represented by literature data. The subjects do not show associated disorders such as impairment of object recognition. The cumulation segregation ratios are compatible with a simple autosomal dominant mode of inheritance. Linkage analyses have been started and will be reported.

P0827. Haplotype and linkage analyses in twelve XLRP Spanish families

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X-linked retinitis pigmentosa (XLRP) is regarded as being the most severe form of RP because of its early onset and its rapid progression, which leads to legal blindness before the age of 25. Four XLRP loci have been demonstrated (RP2, RP3, RP23 and RP24), but only two genes have been cloned so far (RP2 and RPGR). The RP2 locus accounts for 20% of the XLRP forms and the RP3 locus (RPGR) is the most prevalent with 70-80% of cases. The other two loci are very rare.
Haplotype and linkage analyses were carried out in twelve Spanish XLRP families. Six dinucleotide repeat microsatellite markers and one RFLP spanning the RP2-RP3 region, plus an additional microsatellite strongly linked to RP24 were used. An ABI PRISM 310 Genetic Analyzer (*Applied Biosystems*) was the tool of choice for the genotyping process.
After haplotype analysis we could rule out RP24 in 7 families, RP2 in 1 family and RP3 in another family. We found no informative meioses for RP24 in 5 families and for RP2/RP3 in the vast majority of cases. Excluding obligate carriers, we found 14 female carriers of the disease, 12 non-carriers and 5 non-informative females, out of 31 women at risk of being carriers.
In conclusion, this seems not to be an informative method of distinguishing between the main XLRP loci, but it is worth doing because it permits a rapid and effective early detection of carriers, which is of paramount importance for genetic counselling.

P0828. Expression profiling in patients with complex IV respiratory chain deficiencies

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In patients with respiratory chain deficiencies expression profiling of genes involved in mitochondrial biology should provide insight into regulatory mechanisms and possibly affected pathways. To this end we have designed a DNA microarray covering ~1000 human cDNAs which are known to encode mitochondrial proteins (~400) or which represent candidate genes (~600) according to homology criteria. PCR products of these cDNAs were arrayed onto nylon filters and hybridised with radioactively labelled cDNAs from patients fibroblasts and age matched controls. Characterisation of expression patterns was performed in nine patients with biochemically defined complex IV deficiencies, including patients with mutations in SURF1 (2), SCO2 (3), mt tRNASer (2) or no mutations in any of these genes (2).
At least 50% of the sequences investigated showed background level signals indicating low expression of the corresponding genes. However, significant differences between control group and patients were observed with preponderance of a substantial downregulation (10-30 fold) of at least 5% of the genes, including subunits of the respiratory chain complexes e.g. COX1, COX2, COX3, H+-transporting ATP synthase proteins 5A1, 5B and 8 as well as proteins involved in mitochondrial protein synthesis such as transcription elongation factor TUFM and numerous ribosomal proteins. This suggests that on the transcriptional level downregulation of oxidative phosphorylation rather than overcompensation may be a common phenomenon in mitochondrial disease. Thus DNA microarrays may help to classify patients with mitochondrial dysfunction, providing both a diagnostic tool and a way to map mitochondrial disease genes.

P0829. Molecular cloning of a de novo reciprocal translocation t(4;12)(q26;p12) in a patient with Andersen syndrome (periodic paralysis and long QT syndrome)

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Most paroxysmal neuromuscular and cardiac disorders are channelopathies. Andersen syndrome is a condition in which cardiac arrythmias (long QT) and periodic paralysis are associated, thus representing a powerful tool to identify possible common pathways. A de novo reciprocal translocation t(4;12)(q26;p12) was detected in one patient with Andersen syndrome. Both parents were unaffected. Several long QT syndromes (LQT) have been defined. The LQT4 gene maps to 4q25-q27 ; the critical region extends over 18 cM and the gene remains unknown. Colocalization of the 4q26 breakpoint with the LQT4 region suggested that both syndromes could be sustained by the same gene.
FISH experiments showed that the breakpoint at 4q26 lied in a 120-kb area entirely comprised within the LQT4 critical interval. PAC and cosmid clones were identified that encompassed the breakpoint. All clones are situated within a gap of the human genome sequence KCNJ2 (chr.17) was identified as one Andersen syndrome gene while this analysis was underway. A previously reported missense mutation C880T was detected in the patient's DNA but the same mutation was

also detected in her father's DNA. The father was unaffected and even had a normal muscular biopsy. These results indicated that, at least in one case, the C880T mutation might not be pathogenic. The translocation bored by the patient could alter the LQT4 gene, which in turn would generally act as a modifier gene in Andersen syndrome. Consistent with this latter hypothesis is the fact that the LQT4 locus could contribute the QT-interval variability (Busjahn et al, 1999).

P0830. Molecular genetic analysis of LQT syndromes in Russian families

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Congenital long QT syndrome (LQTS) is an inherited cardiac disorder characterized by a prolonged QT interval (rate-corrected QT>440 ms, syncope due to polymorphous ventricular arrhythmias ("torsade de pointes") and high risk of sudden death. At least the six genes, when mutated, produce this phenotype: KCNQ1 (LQT1), HERG (LQT2), SCN5A (LQT3), KCNE1 (LQT5), KCNE2 (LQT6).
Here we present the results of genetic screening (PCR-SSCP-analysis combined with sequencing of abnormal conformers) 2, 4, 5, 6, 7, 14 exons of the gene KCNQ1, exons 2, 6, 7, 10 of the HERG, minK and MiRP1 genes. We have screened 60 unrelated families (132 patients with Romano-Ward syndrome and 3 patients with Jervell and Lange-Nielsen syndrome). Diagnosis was confirmed using criterias according Schwartz et al. (1993).
We have identified 32 mutations in 35 unrelated families and 3 SNP. Distribution of mutations among these 4 genes was followed: 22 in KCNQ1(68,7%), 6 in HERG (18,7%), 3 in minK (9,3%) and 1 in MiRP1(1,9%) genes. Missense mutations represented the majority of genetic defects (90,6%). Other abnormalities were splice errors. We identified 17 novel mutations, the others have been reported. We don't found any mutations in 2 and 10 exons of HERG although it consist about 25% of all known mutations in this gene by published date. The W585C substitution in 7 exon of HERG and the V107I substitution in MinK were detected in the same proband with LQT1 and LQT2 both clinical phenotype.

P0831. Part of the *GLI3* transcripts with premature termination codons undergo nonsense mediated mRNA decay

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Chromosomal aberrations as well as different types of point mutations located throughout the coding exons of the *GLI3* gene are causing distinct morphopathies associated with polydactyly (GCPS, PHS, PPIV, PAPA/B). Most of the point mutations result in the introduction of premature termination codons (PTC). To establish a genotype-phenotype correlation in *GLI3* associated morphopathies a functional role has been invoked for different truncated protein products.
To test if GLI3-mRNA molecules with truncating mutations in different positions are retained for translation we determined the amount of mutant GLI3-mRNA relative to the wild type transcript in patient derived fibroblasts lines using the ARMS (Amplification Refractive Mutation System) test and differential restriction following RT-PCR. Transcripts with PTCs at position 309 or 366 of the cDNA were present at lower levels (20-40%) compared with the allelic wild type transcript. In contrast, transcripts with a PTC located in the last *GLI3* exon or carrying a silent amino acid exchange were present at equal levels compared with their wild type counterparts. These data are in line with previous observations that only transcripts with PTCs upstream of the last intron-exon junction undergo nonsense mediated mRNA decay (NMD).
Haploinsufficiency due to NMD might occur in the 56% of *GLI3* PTC mutations located in the first 14 exons associated with all types of *GLI3*-morphopathies, including PHS. Different mechanisms, like protein instability or functional haploinsufficiency must be invoked for PTC mutations in the last *GLI3* exon that accounts for about half of the coding capacity of the full-length GLI3 protein.

P0832. High incidence of Arg 531→Cys mutation in the factor VIII gene among hemophilia A patients from Republic of Bulgaria

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Hemophilia A is an X-linked bleeding disorder affecting approximately 1 in 5.000 males. It is caused by mutations in the factor VIII gene, leading to a deficiency of factor VIII, a cofactor for the activation of factor X by factor IXa. Factor VIII gene is located on Xq28, spanning 186 kb. It consists of 26 exons and encodes a 9 kb mRNA.
A variety of molecular defects are identified as a cause of hemophilia A. An inversion in intron 22 is the cause of 45% of severe cases with hemophilia A. Most of the other molecular defects in the factor VIII gene are point mutations, that are peculiar to the individual families. Using SSCP as a screening method, we have identified an Arg 531→Cys (CGC→TGC) point mutation in exon 11 in six out of 60 (10%) unrelated patients with mild to moderate hemophilia A patients from the Republic of Bulgaria. The mothers of all the patients were found to be carriers for this mutation.
The Arg 531→Cys amino acid substitution is relatively rare mutation. It was described for the first time in 1991 by Higuchi et al. (Proc Natl Acad Sci USA, 88:7405-09; 1991) in two patients of a Japanese family. According to the database (http://europium.csc.mrc.ac.uk) this mutation has been observed in 12 out of 2.000 unrelated patients. None of our 50 hemophilia A patients from the Republic of Macedonia had this mutation. This is the first observation that this point mutation has a high prevalence among one ethnic group.

P0833. Nonsense-mediated mRNA decay explains protein S deficiency in patients carrying a PROS1 mutation that introduces a premature stop codon

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This study was designed to analyse the effect on mRNA expression of 11 protein S gene (PROS1) mutations that segregate with protein S (PS) deficiency in Spanish thrombophilic families. Two of the mutations are missense (L-27H and M599T); 5 are nonsense (E19X, Q238X, S293X, R410X, W465X), two are frameshift deletions (333,334delTG and 1877delT); one alters a splice site (1301+5G>A) and the last one is splice site and missense (V46F or 404,405AG>GT). PROS1 cDNA fragments covering exons 1 to 15 were obtained by RT-PCR from platelet-derived patientsí mRNAs and analysed by DNA sequencing. The results obtained were confirmed by the analysis of transcribed polymorphisms. cDNAs corresponding to both the normal and the mutated alleles were obtained from the heterozygotes for L-27H, M599T, 333,334delTG and 1301+5G>A. On the contrary, cDNAs corresponding only to the normal allele, which indicated the absence of transcripts from the mutation, were obtained from all 5 heterozygotes for nonsense mutations, as well as from the 1877delT and the V46F heterozygotes. From these results we conclude that: 1) The main cause of quantitative PS deficiency associated to mutations which generate a premature stop codon is not the synthesis of a truncated protein but the exclusion of the mutated allele due to nonsense-mediated mRNA decay. 2) V46F or 404,405AG>GT allele exclusion indicates that an abnormal splicing of intron 3, with the generation of a stop codon, is the most likely cause of PS deficiency associated with this "missense" mutation.
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P0834. DNA Analysis of Puerto Rican albino patients suspected of having Hermansky Pudlak Syndrome

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The most common form of albinism in PR is the Hermansky Pudlak Syndrome (HPS). A 16-bp duplication in exon 15 of the first HPS gene characterised (HPS1) was found in a large group of PR HPS patients. A third gene for HPS (SUTAL or HPS3) was reported to

cause additional cases in Central PR due to a 3,904-bp deletion. We have screened 137 Puerto Rican patients with albinism for the HPS1 16 bp duplication and found that only 48.2% of the patients were homozygous for the duplication, 44.5% were negative, and 7.3% were heterozygous for the HPS1 PR mutation. We have examined 71 HPS1 duplication- negative or heterozygous PR albino patients for mutations in other albinism genes and found that 6 of these patients were homozygous for the G47D mutation of the TYR gene and 17 were homozygous and one heterozygous for the HPS3 PR deletion. Out of 1,416 PR newborns, only 25 (1.76%) were heterozygous and one was homozygous for the HPS1 duplication mutation. The carrier frequency for the HPS1 duplication in Northwest PR was 12 out of 301 newborns (1:25), whereas 10 out of 630 newborns (1:63) carried the HPS3 gene deletion. The PR HPS1 16 bp duplication accounts for close to half of the albinism cases in this cohort; the HPS3 deletion was responsible for 12.4% the G47D tyrosinase mutation only accounts for 4.4%; and 35.0% require further analysis. NIH-NIGMS grants S06-GM08224 and R25-GM61838 and RCMI award G12RR03051 supported this study.

P0835. KIF1B gene study in the large Russian CMT2A family

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Charcot-Marie-Tooth disease (CMT) is the most common human hereditary neuropathy affecting both motor and sensory peripheral nerves. CMT is divided into two large types: demyelinating CMT1 and axonal CMT2, both are genetically heterogeneous. Recently, two genes responsible for CMT2 were identified. These comprise the neurofilament light gene for a CMT2E locus on 8p21, and the kinesin like gene KIF1B for a CMT2A locus on 1p36. To date, the only pathogenic KIF1B gene mutation resulting in a Gln98Leu substitution was described in a single CMT2A Japanese family.
Here we report on a large Russian CMT2 family, in which a significant positive LOD score of 3.55 was obtained for markers of the CMT2A locus. All KIF1B exons including flanking parts of introns were analyzed in this family by direct sequencing. No sequence changes resulting in an amino acid substitution or a splice site mutation were found in affected persons. Several intronic polymorphisms, revealed also in normal donors, were found in this family, including CMT patients. This fact allows us to exclude the deletion of the whole KIF1B gene as a cause of CMT in this family.
We propose two hypotheses to explain the present situation: (1) In this family the disease is caused by deletion of a whole exon or by mutations in the noncoding part of the KIF1B gene, which could not be revealed by sequencing. (2) There might be another gene in the 1p36 chromosomal region the mutation of which is responsible for the axonal type of CMT2A neuropathy.

P0836. Severity of Monilethrix associated with a novel promoter polymorphism and the known pathogenic mutation in the helix termination motif of the hair cortex keratin gene, hHb6, in two Indian families.

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Monilethrix is a rare autosomal dominant hair defect with variable expression, characterized by hair fragility and follicular hyperkeratosis. The Pathogenic mutations reported in diseased condition are restricted to helix initiation motif and the helix termination motif of the human basic hair keratin genes, hHb1 and hHb6, located on 12q13, however, the severity of disease and hair dystrophy varies in monilethrix individuals which is unexplainable with the same mutational background. There is no report available for the status of the promoter and intron regions of these genes in the disease condition. Here in this study, two unrelated families with 22 individuals were clinically diagnosed with monilethrix in 15 of its members. In order to investigate the pathogenic mutation in keratin gene, the Promoter region & the gene segment responsible for encoding the helix termination motif of keratin gene, hHb6, was PCR amplified and subjected to SSCP. The variant bands obtained were cloned and sequenced. We observed an association of the disease causing mutation, E413K, in hHb6 gene in 13 out of 15 patients in both the families. We also detected a novel polymorphism in the Promoter and intron 7 of hHB6 gene. Initial observation indicates that both E413K and promoter polymorphism when present together contribute to the severity of the disease and hair dystrophy, an observation not reported earlier.

P0837. Molecular analysis ot hepcidin gene in Italian patients with hereditary hemochromatosis.

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Hereditary hemochromatosis (HH) is the most common genetic disorder in the Caucasian population, particularly frequent in individuals of northern European origin of Celtic ancestry. It is caused by excess absorption of dietary iron by the intestinal mucosa leading to progressive iron deposition in the parenchimal cells of several tissues, particularly of liver. Although the majority of HH cases are associated with mutations in HFE, other genes, some of which already identified, play a role in primary iron overload. Hepcidin, encoding an antimicrobial peptide synthesised in the liver, has been recently proposed as a novel candidate gene involved in the development of HH. In fact , hepcidin gene it is overespressed in mice, subjected to iron overload. Furthermore, Usf2 gene knockout mice, develop extensive iron overload accompanied by lack of hepcidin gene expression.
We performed hepcidin gene mutation analysis in 10 Italian patients with HH, negative for C282Y, H63D or other less frequent HFE mutations and for Y250X in TFR2. Sequencing of the coding region, 5’ and 3’UTRs didn’t reveal the presence of any alteration of the hepcidin gene in the screened patients. Our results exclude, at least in our patients, that hepcidin gene mutations are related to iron overload.

P0838. Clinical and genetical differences between adult primary pulmonary hypertension and the infantile form of the disease.

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Background: Primary pulmonary hypertension (PPH) is a rapid progressive disease with remodeling of the small pulmonary arteries which occurs at any age. In the families with autosomal dominant inherited PPH of adults mutations of the bone morphogenic protein receptor 2 (BMPR2) gene have been identified. The genetic cause of PPH in children who are often severely affected remains unknown.
Methods: We studied 42 relatives of 5 children with PPH, aged 8 to 32 months at diagnosis. PPH was confirmed by right heart catheterisation in all patients. The pulmonary artery systolic pressure (PASP) was estimated by Doppler-echocardiography at rest and during exercise in all family members. A screening of BMPR2-gene was performed by SSCP and DHPLC. Linkage analysis was carried out with markers of chromosome 2.
Results: In all children but one both parents and relatives out of both paternal and maternal branches showed abnormal pulmonary artery pressure responses during exercise. None of the children or their relatives showed mutations of the BMPR2 or Alk1-gene. We found no linkage to chromosome 2q31-33 in all families.
Conclusion: PPH in the 5 children and their families appeared to be transmitted by an autosomal recessive mode of inheritance without evidence of BMPR2-mutations. Therefore, we hypothesise that PPH in children (IPPH) might be genetically different from PPH in adults.

P0839. The "Reunion" mutation is a complex rearrangement of the CYP21 genes and associated wth the 2/3 of classic forms of 21-Hydroxylase Deficiency in the Island

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Incidence of the 21-OH deficiency (21OHD) was 1 in 14000 live births in the world except in Alaska and Reunion island. In this island of the Indian Ocean near Madagascar, the incidence of SW forms was found higher (1:2141) by neonatal screening (1978-1988). We have determined the molecular defects of the CYP21 genes in more than thirty 21OHD. A new mutation with a high incidence (69% SW-chromosomes) was identified and carefully characterized. Southern blotting studies have showed an unusual Taq-1 pattern suggesting a reverse gene conversion CYP21P pseudogene to CYP21 gene without deletion. Nevertheless using PCR methods associated with the entire sequencing of the genes, this rearrangement did not result in two normal CYP21 genes, but in two duplicated CYP21/CYP21P/CYP21 hybrid genes. The presence of multiple mutations predicted no 21-hydroxylase activity. As all patients homozygous for this mutation have a SW form, a good relationship between genotype and phenotype has been observed. This mutation was found in a genetically homogeneous isolate called French small white, but not in the other ethnic populations of this island (Asian, Indian,....). The incidence of the other mutations was similar to this observed in Caucasian populations. Reunion mutation seems to originate from a single common ancestor who was among the settlers of the island. In our large French population (1732 classic CAH chromosomes), each time that this mutation has been detected, the patients were from Reunion Island. A screening of this mutation should be done to improve the genetic counseling of 21OHD in this island.

P0840. Functional studies of SLC7A7 mutations found in patients affected by Lusinuric Protein Intolerance

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Lysinuric protein intolerance (LPI, MIM 222700) is an autosomal recessive defect of cationic amino acids transport (lysine, ornithine, arginine, namely CAA), caused by mutations of the SLC7A7 gene. CAA transport is mediated by y+L system, which is exerted by a heterodimer consisting of the 4F2 heavy chain (4F2hc) and SLC7A7 (4F2hc/SLC7A7). We have identified 16 causative mutations of the SLC7A7 gene in LPI patients, originating from Italy, Tunisia, Greek, Algeria, Pakistan and Japan. We examined nine of these mutations (M1L, M50K, F152L, T188I, S386R, W242X, Y457X, 346delGGA, and 1425delTTCT) to assess the arginine transport function by co-injection of each SLC7A7 mutant with 4F2hc cRNA in X. laevis oocytes. Eight mutations (M1L, M50K, T188I, S386R, W242X, Y457X, 346delGGA and 1425delTTCT) abolish the arginine transport as compared with the 'wild-type' control (4F2hc/SLC7A7). Another mutation, F152L, reduces the arginine transport of about 10% only. We then performed triple injections (cRNA ratio 1:0.5:0.5) of 4F2hc/F152L+346delGGA, 4F2hc/wt SLC7A7+346delGGA, and 4F2hc/wt SLC7A7+F152L which resemble the genotypes of one patient and his parents. The arginine uptake was abolished when the 4F2hc/SLC7A7+346delGGA or the 4F2hc/F152L +346delGGA were injected into the oocytes. We speculate that the 346delGGA mutation might have a dominant negative effect. This correlates well with the severe clinical LPI phenotype observed in the patient. The 346delGGA mutation removes a glutamic residue from the protein though letting a correct reading frame of the protein. Biochemical investigations of the parent carrier of this mutation are in progress.

P0841. Molecular analysis of 737 mutations in the human LDL receptor gene database (UMD-LDLR).

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To date, 820 mutations have been identified in the LDLR gene, encoding the low-density lipoprotein receptor, in subjects with Familial Hypercholesterolemia. Although genotype/structure-function correlations have been substantially investigated, genotype/phenotype correlations have not been explored. Thus, we have compiled a database containing standardized data for each LDLR mutation, and developed the software that provides sorting tools and allows optimized multicriteria research [http://www.umd.necker.fr]. The analysis of the 737 point mutations in the UMD-LDLR database gives the following information: [1] 58% of the mutations are missense, and 16% occur in CpG dinucleotides known to be mutational hot spots; [2] although widely distributed throughout the gene, there is an excess of mutations in exons 4 and 6 (ligand-binding repeats) and 7 (EGF-like repeat); [3] there is a deficit of mutations in exons 10 and 13 (EGF-precursor-like), 15 (O-linked-sugar), 16 (transmembrane), 17 and 18 (cytoplasmic); [4] 49% of the small deletions occur between repeated sequences and can be explained by the slipped-mispairing model described by Krawczak and Cooper; [5] 67% of the mutations in the ligand-binding domain affect conserved amino-acids involved in LDL binding; [6] the functional data available for 183 (29%) mutations indicate 38% of class 2B (transport defective) and 33% of class 1 mutations (null alleles).

P0842. The genetic IP defects: molecular analysis of NEMO gene and NF-kB related genes.

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In collaboration with International IP Consortium (IPIF) we recently demonstrated that 78% of patients of Incontinentia Pigmenti (IP; MIM 308310) show amorphic mutations in NEMO/IKKg gene. The complete loss of NF-kB activation is lethal for males during embryogenesis but females can survive, owing to mosaicism as a result of X-inactivation. Moreover, it has been reported that hypomorphic mutations in the NEMO gene lead to an Ectodermal Dysplasia, Hypohidrotic with Immunodeficiency (ED-ID; MIM 300291). In human, these mutations affecting NEMO gene impair but do not abolish NF-kB signaling resulting in two related syndromes that associate specific developmental and immunological defects. Among 357 patients analyzed by the IPIF, 84 are collected in our lab; 56 of them carrying the D4-10 deletion, 1 show the E57K mutations and 4 are carrier of new small mutations. Mutational analysis by DHPLC revealed that 23 patients, 1 familial and 22 sporadic cases, do not exhibit mutations in NEMO gene, although a typical IP phenotype has been ascertained on clinical presentation. Since multiple pathways impinge on the NF-kB transcription system, it is conceivable that combinations of mutations in the upstream and downstream genes could cause a phenotype similar to that produced by specific defects in NEMO. For this purpose, we are identifying and characterizing regulatory regions of the NEMO promoters and search for mutations in IP and ED-ID patients, which still lack a molecular diagnosis. In the meantime, we are searching for other genes involved in NF-kB action that could affect NEMO function

P0843. In search of the forgotten exon of the human crumbs homolog 1 and its implication in LCA.

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To identify the gene responsible for Leber congenital amaurosis in a very large consanguineous family originating from Palestine, a genome wide search for homozygosity was undertaken. We found evidence for homozygosity for markers of the 1q31 region. Recombination events in two affected patients allowed reducing the genetic interval containing the disease-causing gene between D1S1723 and D1S2668. The CRB1 gene was found to map in this 4 cM interval. However, prior to this genome wide search for

homozygosity, a screening of the six already known LCA-causing gene including CRB1 was performed but no mutation was found. The mapping of the disease-causing gene in this family prompted us to consider that the mutation might lie in a forgotten exon of the CRB1 gene. Subsequently, we screened the Human Genome Working Draft in order to identify novel CRB1 exons. This study revealed that a twelfth exon lies in the 3' end of the gene. The sequence of this exon revealed that the disease in this family is accounted for by a homozygous 10 bp deletion leading to apparition of a premature Stop codon. Further study of this exon in 80 unrealted LCA patients was therefore undertaken.

P0844. Dominant X-linked RP is frequently accounted for by truncating mutations in the exon ORF15 of the RPGR gene.

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Purpose: To determine whether dominant X-linked retinitis pigmentosa (DXLRP), a condition previously described as different from the recessive X-linked RP (RP3), are due to mutations in the retinitis pigmentosa GTPase-regulator (RPGR) gene.
Methods: The RPGR gene was screened for mutations in fourteen X-linked RP families with severe expression in carrier females.
Results: In 9/14 families, 8 different null mutations were found in the RPGR ORF15 exon. In the 5 remaining families, no mutation was found. Nevertheless, linkage analyses confirmed the localisation of the gene in all families at the RP3 locus, suggesting that in these last 5 families the RPGR mutation might be overlooked.
Conclusions: We report here on the identification of null RPGR alleles in patients affected with DXLRP. In this retinal dystrophy, both males and females display minimal inclusion criteria for RP. Although, the age at onset of the disease in females is delayed compared to males (20-40 years *versus* 10-20 years, respectively) the visual impairment, the fundus alteration and the visual field reduction can be as severe in heterozygous females as in hemizygous males. In these females who's ERG is non-recordable, no preferential X-inactivation was observed. It would be extremely interesting to know the exact phenotype of females harbouring truncating mutations in the RPGR exon ORF15 in the XLRP families recently reported. Indeed, if some of the women were more severely affected than what is usually described for carrier females in recessive X-linked RP we would have to consider RP3 as an incomplete dominant X-linked disease.

P0845. Elucidating the molecular basis of triallelism in Bardet-Biedl syndrome.

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Bardet-Biedl syndrome (BBS) (OMIM: 209900) is a rare genetically heterogeneous disorder characterized primarily by obesity, retinal dystrophy, polydactyly, hypogenitalism, learning difficulties and renal malformations. BBS has been considered historically an autosomal recessive trait, on which premise six loci have been mapped in the human genome. Nevertheless, mutational and genetic analyses of the first three cloned *BBS* genes, *BBS2*, *BBS4* and *BBS6* suggest that BBS may also exhibit a complex mode of disease transmission, requiring at least three mutations in two genes to manifest the phenotype (triallelic inheritance). In an effort to elucidate the molecular basis of triallelism, we have reasoned that either the different BBS proteins are part of the same multisubunit protein complex, or that they operate in discrete yet complementary pathways. Based on these hypotheses, we are conducting coimmunoprecipitation and immunohistochemical studies to ascertain the cellular localization and potential interaction between the three known BBS proteins. We present data from coimmunoprecipitation assays performed by transiently co-transfecting HEK293 cells with Myc- and HA-tagged BBS proteins, that suggest that the three BBS proteins do not interact directly. Furthermore, cellular localization studies indicate that BBS2 and BBS4, but not BBS6, may be localized in the same cellular compartments, suggesting that the molecular basis of triallelism in BBS may be due to cellular rescue by alternative pathways. Elucidation of the molecular etiology of BBS and understanding of triallelism may prove important in bridging between Mendelian and complex traits.

P0846. TGFβ 1 screening in Camurati-Engelmann like and others diaphyseal sclerosing bone disorders

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Camurati-Engelmann disease (CED) is a rare autosomal dominant progressive sclerosing diaphyseal dysplasia due to TGFß 1 mutations. On a total of 31 families studied, 3 families have been reported without any TGFß 1 mutation including two families with hyperostosis generalisata with bone striations.
Here, we report on the molecular study of TGFß 1 gene in three sclerosing disorders presenting clinical and radiological similarities with CED. The series include : 1) a CED like disorder observed in a two generation family with three affected patients presenting in addition bone striations. 2) a Ghosal syndrome observed in a large consanguineous family with 2 affected patients characterised by anaemia responsive to corticosteroids and diaphyseal dysplasia but absence of generalised manifestations. 3) two patients with craniodiaphyseal dysplasia.
In all these cases, TGFß 1 was tested by direct sequencing and no mutation was found in the first 6 exons of the coding sequence. These results suggest that at least one other gene is involved in sclerosing diaphyseal dysplasia.

P0847. Detection of mutations in the translation initiation factor eIF2B in a restricted white matter disorder, the CACH/VWM syndrome

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The Childhood Ataxia with diffuse Central nervous system Hypomyelination (CACH) syndrome is an autosomal recessive leukodystrophy clinically characterized by a progressive ataxic diplegia with additional episodes of rapid deterioration following febrile infection or minor head trauma leading to death within a few years after onset. MRI shows a diffuse hyposignal of the cerebral white matter resembling the intensity of CSF (Vanish White Matter (VWM). It has been further characterized as a cavitating leukodystrophy with an increased number of oligodendrocytes with foamy feature. Phenotypic variation with later onset, slow chronic progressive disease with learning and behavioral difficulties have been also reported. We analyzed 27 CACH families (12 familial cases) and found a genetic heterogeneity at the previously reported 3q27 locus (maximum HLOD score=2.67 between markers D3S1618 and D3S3609 and 5 excluded families). Genetic heterogeneity of this syndrome was recently confirmed by the description of mutations in the five subunits of the eIF2B translation initiation factor (eIF2Ba (12q24), eIF2Bß(14q24), eIF2Bg(1p36.23), eIF2Bd (2p23), eIF2Be(3q27)). Complete sequence analysis of RT-PCR eIF2Be cDNA demonstrated a missense mutation in 50% of families not excluded for the 3q27 locus by linkage analysis. All were in exons 3 (60%), 2 (20%), 7 and 4 (10% each). 40% of alleles expressed a preferentially R113H mutation. No genotype were strictly correlated with the severity of the disease, however the 5 homozygous patients for this R113H mutation have a milder form than heterozygous patients for the same mutation.

P0848. GJB2 mutation in Iranians with autosomal recessive non-syndromic sensorineural hearing loss

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Hereditary hearing loss (HHL) is an extremely common disorder. About 70% of HHL is non-syndromic, with autosomal recessive forms accounting for ~ 85% of the genetic load. Although very heterogeneous, the most common cause of HHL in many different world populations is mutations of GJB2, a gene that encodes the gap junction protein connexin 26 (Cx26). This study investigates the contribution of GJB2 to the autosomal recessive non-syndromic deafness (ARNSD) load in the Iranian population. One hundred sixty eight persons from 83 families were studied. GJB2-related deafness was diagnosed in 9 families (4, 35delG homozygotes; 3, 35delG compound heterozygotes; 1, W24X homozygote; 1, non-35delG compound heterozygote). The carrier frequency of the 35delG allele in this population was ~1% (1/83). Because the relative frequency of Cx26 mutations is much less than in the other populations, it is possible that mutations in other genes play a major role in ARNSD in Iran.

P0849. Loss of a chloride channel and a proton pump lead to slightly divergent forms of osteopetrosis in mice and man.

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Chloride channels play important roles in the plasma membrane and in intracellular organelles. ClC-7 is a ubiquitously expressed chloride channel that resides within late endosomes and lysosomes. Mice deficient for ClC-7 show severe osteopetrosis that becomes apparent shortly after birth and retinal degeneration. Although osteoclasts are present in normal numbers, they fail to resorb bone because they cannot acidify the extracellular resorption lacuna. In osteoclasts, ClC-7 is highly expressed in the ruffled membrane that is formed by the fusion of H+-ATPase containing vesicles and that secretes protons into the lacuna. We conclude that ClC-7 provides the chloride conductance required for an efficient proton pumping by the H+-ATPase of the osteoclast ruffled membrane. Comparison with the mouse mutant osteosclerotic (oc), which lacks a subunit of the H+-ATPase, revealed that the retinal phenotype is not secondary to the osteopetrosis but most likely a direct effect of the loss of ClC-7 on the photoreceptors.
As the murine phenotype closely resembles human infantile malignant osteopetrosis, we also searched for mutations in the human gene, CLCN7, in 23 patients suffering from this disease. Indeed, three patients were compound heterozygous for mutations. Some mutations lead to a complete loss of the ClC-7 protein in cultured fibroblasts from the patients. The majority of cases, though, was positive for mutations in TCIRG1, which codes for the a3 subunit of the osteoclast H+-ATPase.

P0850. In the phylogeny, PHEX, whose mutations cause X-linked hypophosphataemic rickets (HYP), occurs concomitantly with the bony fish

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HYP patients suffer from phosphate leakage in the kidney, growth retardation, and abnormal bone and tooth mineralisation. The product of the PHEX gene (phosphate regulating gene with homologies to endopeptidases on the X chromosome) belongs to the M13 metalloendopeptidases family. These enzymes cleave a broad range of small physiologically active peptides. M13 endopeptidases are characterised by 3 catalytic signatures: ^{580}HExxH^{584}, the zinc binding motif, ^{642}ExxxD^{646}, and ^{537}VNAFY^{541}. Since PHEX has only been studied in mammals, the question arises to know whether it participates in phosphate metabolism in organisms lacking bone and teeth. A primary sequence alignment has been carried out and 81 M13 endopeptidases were identified from bacteria to human. Five new evolutionary conserved motifs were detected. One of them, ^{76}PCxxFFxFACxxW^{88}, close to the transmembrane segment, is eukaryotic specific. Four new conserved motifs were localised in the C-terminal region: ^{456}WMxxxTKxxAxxK^{468}, ^{476}VGYP^{479}, ^{602}WW^{603}, ^{746}CxLW^{749}. When superposed with the cristallographic neprilysin (the prototype of M13) structure, the new motifs appear to be concentrated in the proximity of the catalytic signatures. No PHEX orthologue was detected in invertebrates. A zebrafish EST containing the motif I was identified as an M13 endopeptidase sequence. After sequencing the whole clone, all the above motifs were identified, and the protein revealed a high homology with the mammalian PHEX. PHEX definitely appears tightly linked to tissue mineralisation.

P 18. Muscle diseases

P0851. An sporadic case of DMD girl due to a deletion in dystrophin gene and skewed inactivation of normal X chromosome

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Duchenne muscular dystrophy (DMD) is an X-linked inherited disorder characterized by the absence of dystrophin in myofibers, with a prevalence of 1 in 3000 newborn males. Only about 8% of the heterozygote female carriers show clinical symptoms, wich can be as mild as pseudohypertrophy of the calf muscles or proximal limb weakness. In the rare cases of DMD females, cytogenetic studies have shown either the absence of one X chromosome or an X-autosome translocation. However, there are reports of rare manifesting carriers with a normal karyotype but with preferential inactivation of the normal X chromosome.
We present a young girl, an sporadic case, with a DMD phenotype. The inmonocytochemistry with anti-dystrophin antibodies (Novocastra) showed the total absence of this protein in the muscle tissue. The multiplex PCR showed a deletion of exon 3 to exon 44 (both included). The microsatellite analysis using STRs located in the deleted region (STR07A and STR44) demonstrate hemizygosity with paternal origin. The healthy brother present the same maternal haplotype. The pattern of inactivation was studied by differential methylation that exists between the active chromosome and inactive chromosome in the CpG islands of the X-linked genes, by using methylation-sensitive enzymes. The results confirmed a nearly total inactivation of the maternal chomosome.
In spite the difficulty that this kind of families, without affected boy, provides to the molecular analysis of the dystrophin gene, is important to perfoms this kind of stuies in girls with clincial symptoms of myopathy to establish the exact diagnosis of the disease.

P0852. Evidence for autosomal recessive inheritance in the infantile spinal muscular atrophy variant with congenital fractures.

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We report on a female newborn with a severe acute form of SMA, congenital bone fractures, camptodactyly of fingers and toes, bilateral hip dislocation and congenital heart defect, with early lethal outcome. DNA studies showed the absence of homozygosity for a deletion of exons 7 and 8 of the SMNt gene. A new lethal syndrome consisting of infantile spinal muscular atrophy (SMA) and multiple congenital bone fractures in 2 sibs has been suggested in 1991. Recently, another infant with a form of SMA and congenital fractures, was reported, thus validating the suggestion of a distinct and rare form of SMA associated with congenital bone fractures. Autosomal recessive inheritance was suggested in the original report, but no history of consanguinity was noted in the second. X-linked inheritance could however not be excluded since those three affected infants were male. Since our case is a female, an X-linked inheritance can be

excluded. Since she was furthermore born to first cousin parents, it suggests an autosomal recessive inheritance in this rare variant of SMA type 1 with congenital fractures. We further conclude that this SMA variant, with early lethal outcome seems to have a variable clinical expression and that it is probably not linked to 5q.

P0853. Spinal muscular atrophy type 1-3 in Estonian children

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The DNA testing for spinal muscular atrophy (SMA) became available in Estonia in 1997. Since then, 15 children with the clinical diagnosis of SMA have been studied. In 13 cases the diagnosis was confirmed by DNA analysis, in 2 cases the deletion was not found and the diagnosis based on clinical findings, electroneuromyography and muscle biopsy. 6 patients had type 1 (Werdnig-Hofmann), 2 had type 2 and 7 had type 3 (Kugelberg-Welander) SMA. Patients with type 2-3 disease had no complications at birth, those with type 1 SMA were asphyctic at birth, one patient was born with limb fractures. 5 out of 6 SMA type 1 patients had muscle weakness and hypotony at birth already, 1 developed those symptoms by age of 2 months. 5 patients died of respiratory insufficiency in first 6 months, one patient survived to 1.5 years. None of the SMA type 2 patients have ever walked unassisted. All SMA type 3 patients developed clinical features of the disease before the age of 3 years, 4 of them have lost the ability to walk at the age of 8-15 years.
We recommend to perform molecular testing for SMA in all children who are born in asphyxia and muscle weakness.

P0854. Microcephaly-cardiomyopathy syndrome: further deliniation of the phenotype

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Winship et al. reported South African sibs with a combination of microcephaly, dilated cardiomyopathy and minor dysmorphic features in 1991. The cardiomypathy had resolved in the older child by the age of three years, and had markedly improved in the younger child on anticardiac failure therapy. The microcephaly was severe, and both children showed severe global developmental delay. The dysmorphic features were described as cupping of the outer helix of both pinnae, fifth finger clinodactyly and sandal gaps on both feet.
Kennedy et al. reported a nine year old girl with microcephaly, severe developmental delay and a dilated cardiomyopathy which had resolved at seven years of age. This patient had a sloping forehead, downslanting palpebral fissures, a narrow palate, small ears and a big sandal gap. Magnetic resonance imaging of the brain was normal. All three children initially presented with cardiac failure, at the ages of two months, five months and neonatally respectively. There was no consanguinity in either family.
We report another case with microcephaly and a dilated cardiomyopathy, but without soft dysmorphic features, which suggests that these are more likely to represent coincidental familial traits.

P0855. Genetic analysis of hypertrophic cardiomyopathy in 12 Croatian families

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Hypertrophic cardiomiopathy (HCM) is a genetically and clinically heterogeneous myocardial disease that in most cases familial and transmitted in a dominant fashion. More than 140 different mutations in 11 sarcomeric genes have been described to date. The most frequently affected gene codes beta-myosin heavy chain (MYH 7) (35-50%). Previous genotype-phenotype correlation studies have shown that mutations carry prognostic significance (R403Q, R719W and R719Q mutations were identified as highly malignant defects). We analysed MYH 7 in 14 patients (7 female and 7 male), members of 12 unrelated families, with HCM. The median age of patients at the time of diagnosis was 11,2 years. In 8 patients dominant inheritance was strongly suggested on the base of family history. Mutation analysis of MYH 7 was carried out for exons 8, 9, 13, 15, 16, 19, 20 and 23. Thirty-nine known mutations (9 malignant including R403Q, R719W and R719Q); 36 substitutions, 2 deletions and 1 insertion were analysed. The mutations were detected using mutation specific restriction enzyme assays and oligonucleotide sequencing. No mutation has been found in the analysed patients. The non-existence of malignant mutation amongst the analysed patients, especially those with a positive family history, it difficult to explain on the basis of published studies till 2000. The research carried out by Ackerman in 2001, has shown for the first time very low incidence of malignant mutations, less than 1%, what also confirm the results of this study.

P0856. The possible modifier effect of mitochondrial DNA defect in familial hypertrophic cardiomyopathy causally linked to beta-MHC gene mutation.

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Two pathological mutations, one in mitochondrial DNA (mtDNA) and one in a nuclear gene were identified in a large Italian family with hypertrophic cardiomyopathy (HCM). Patients carrying both heteroplasmic T9957C (Phe→Leu, COX III subunit) mutation of the mtDNA and Lys450Glu mutation of the beta Myosin Heavy Chain (beta-MHC) were affected by HCM and developed congestive heart failure, while patients carrying the nuclear defect, but not the mtDNA defect, did not develop heart failure. The mtDNA mutation alone was not associated to clinical phenotypes, any type. Among patients with congestive heart failure in optimal medical treatment, all those who underwent or are awaiting for hert transplantation had higher amount of mutant DNA than those who are clinically stable. The cosegregation of congestive heart failure with a heteroplasmic mtDNA defect in our large family with HCM, causally linked to a beta-MHC gene mutation, indicates that mtDNA defects are possible candidates to the role of modifiers, influencing the evolution of the disease toward heart failure.

P0857. Risk Estimates For Genetic Counselling In Myotonic Dystrophy

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The combination of clinical variability, anticipation and parent of origin effects creates considerable difficulties in providing accurate risk estimates for relatives at risk for myotonic dystrophy, while the recent recognition of a second locus (PROMM/DM2) is an additional factor. Available genetic risk data from both the early and recent literature and from personal studies are reassessed and combined to give a series of estimates suitable for genetic counselling of myotonic dystrophy families. In particular it is relevant for presymptomatic testing that the risk of a clinically normal, adult first degree relative carrying the myotonic dystrophy (DM1) mutation is unlikely to exceed 10%.

P0858. Multiple consanguinity in a large Azorean family affected with Spinal Muscular Atrophy type I: Implication in genetic counselling

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In small populations, consanguinity is an important factor in the appearance of recessively transmitted hereditary diseases, like spinal muscular atrophy (SMA). This disease is classified into three types, one of which is the early infancy severe SMA type I. About 95% of the affected individuals have homozygous deletions of exons 8 and/or 7 in the *SMN1* gene.

Here, we describe a multiple consanguineous kindred from the Azorean island of São Miguel, Portugal, with one child (proband) affected with SMA type I. This child, who died before 1, was the first offspring of a close consanguineous marriage (inbreeding coefficient, F= 0.0703). The ascending genealogy of the proband shows that her parents and one set of her great-grand parents were both first cousins. In addition, the extended genealogical analysis revealed another consanguineous marriage (paternal proband's uncle and his wife) with a lower value of relationship (F= 0.0195), although relevant in terms of genetic risk to offspring. No homozygous deletion of *SMN1* and *NAIPt* genes were found in the proband, indicating that she probably has a rare mutation. However, haplotype analysis shows homoallelism for five closely linked polymorphic markers. Considering the familiar consanguinity, the proband homoallelism suggests identity-by-descent at SMA locus. The proband's uncle and his wife are both carriers for the same haplotype, thus each offspring has a risk of 25% to be affected with SMA type I.
In conclusion, this study shows that the extended genealogical prenatal investigation and genetic counselling are very informative in families carrying rare recessive genes. (lmv.hospdelgada@mail.telepac.pt)

P0859. Gene expression profiling analysis shows possible signal transduction pathways leading to cardiac structural changes in left ventricular hypertrophy of renal failure

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By gene expression profiling, we have investigated signaling molecules which obviously take in a major role in re-organizing the cytoskeleton, the extracellular matrix, and the capillary density in hearts of rats with renal failure. Male Sprague-Dawley rats, which were subjected to sub-total nephrectomy (SNX), served as a model system for a gene expression profiling analysis. Poly(A)+ RNA from the hearts of SNX animals and from sham-operated rats (SHAM) as a control, was labeled and hybridized with Rat UniGene filters containing about 27.000 gene and EST sequences (Bento Soares, Univ. of Iowa). Phosphoimaging and software analysis revealed substantial changes in gene expression in SNX animals compared to SHAM: Not only integrin a1 and b1 as central players, but also genes downstream of the integrin signaling pathway, like calreticulin, rac protein kinase a, rho A and rho B have been shown to be up-regulated in SNX animals. Rho protein again, might be causal for the stimulation of the expression different enzymes of the phosphatidylinositol pathway up to cardiac dynein and actin, which also has been shown by our experiments. Therefore, the gene expression profiling experiments discussed here not only allow us to describe genes involved in activation and expansion of the non-vascular interstitial tissue in uraemic animals, like timp3, tgf-b1, osteonectin, paxillin, and laminin a1, and linker molecules like plectin, catenin, cadherin and ICAM. These experiments also make it possible to find central signaling molecules responsible for the pathomechanisms involved in cardiac structural changes upon renal failure.

P0860. Towards the identification of molecular pathways underlying ADAM 12's role in myogenesis

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The ADAMs (A Disintegrin And Metlloprotease) are a recently described family of cell membrane anchored glycoproteins. During mouse development ADAM 12 is expressed in several tissues including muscle. Postnatally, the expression of ADAM 12 in the muscle ceases and only reappears transiently during muscle regeneration.
To study the role of ADAM 12 in vivo, we generated transgenic mice that overexpress ADAM 12 in the muscle beyond embryonic life. These mice showed accelerated regeneration following acute injury. Interestingly, when ADAM 12 transgenic mice were paired with dystrophin-deficient mdx mice, the enhanced expression of ADAM 12 resulted in a decrease of muscle cell necrosis and inflammation and a more than 50% reduction in serum creatine kinase.
In order to begin to decipher the molecular mechanism of ADAM 12's action in muscle, we examined on DNA microarray chips the expression of 12500 genes in ADAM 12 transgenic mice and their littermate controls. Three independent experiments gave similar results; in particular, compared to their littermate controls, the transgenic mice showed an increase in the expression of myogenin, myosin, troponin and acetylcholine receptor which are implicated in muscle development and structure. Besides, P21 cycline-dependent kinase inhibitor, which is implicated in cell survival showed an increased expression in transgenic mice. The analysis of mdx and ADAM 12/mdx microarray experiments are underway.

P0861. Muscle specific alternative splicing of myotubularin-related 1 gene is impaired in DM1 muscle cell cultures

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The myotubularin-related 1 (MTMR1) gene belongs to a highly-conserved family of phosphatases, which includes hMTM1, mutated in X-linked myotubular myopathy, a severe congenital disorder that affects skeletal muscle, and hMTMR2, mutated in Charcot-Marie-Tooth type 4B, a recessive demyelinating neuropathy with a specific Schwann cell pathology. We and others recently showed that the MTM1 gene product, myotubularin, is a potent phosphatidylinositol 3-phosphate (PI(3)P) phosphatase. We now demonstrate that this function is conserved amonsgt other members of the family, in particular MTMR2 and MTMR1 proteins. Whereas no mutations in the hMTMR1 gene have been associated with a human disorder so far, this gene, that arose from an ancient hMTM1 duplication and is adjacent to it in Xq28, may share some biological functions with MTM1, as the corresponding proteins are 57% identical. We investigated whether MTMR1 could play a role in myogenesis by analysing its expression pattern during muscle differentiation both in vitro and in vivo. We have identified 3 novel coding exons in the MTMR1 intron 2 that are alternatively spliced, giving rise to at least four mRNA isoforms. One of the transcripts is muscle-specific. We analysed MTMR1 alternative splicing in muscle cells derived from patients with congenital myotonic dystrophy (cDM1), a disease with RNA splicing disturbances. We have found a reduction of muscle-specific isoform levels and the appearance of an aberrant MTMR1 transcript in cDM1 myotubes in culture. Our results suggest that MTMR1 plays a role in muscle formation and represents a novel target for aberrant pre-mRNA splicing in myotonic dystrophy.

P0862. The neuron-specific RNA binding proteins CELF3 is a component of the DMPK mRNA-associated ribonucleoprotein complex: implications for myotonic dystrophy

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Cognitive impairment is a common finding in congenital myotonic dystrophy type 1 (DM1), being also associated with late onset DM1. The relative independency from the muscular deficits of this DM1 psychiatric feature could be explained on the basis of a molecular perturbation selective of the CNS, which is at the moment completely unknown. A CTG microsatellite expansion in the 3' UTR of the DMPK mRNA is the cause of DM1, and a substantial body of evidence is indicating that the majority of the clinical features of DM1 are consequent to a still undefined perturbation in the cellular

ribonucleoprotein infrastructure, due to an aberrant interaction of RNA binding proteins with the expanded DMPK mRNA. Here we further characterize a family of six human RNA binding proteins, ortholog of the Drosophila Bruno translational repressor, which are component of the DMPK mRNA-associated ribonucleoproteins. Two of these proteins, CELF3 and CELF5, display a strictly neuron-specific pattern of expression. CELF3 appears to be extremely well conserved in evolution and selectively expressed in certain regions of the mouse adult brain, while its developmental expression pattern in the mouse is indicative of a role in brain formation. Therefore, a perturbation of localization or activity of CELF3 could be involved in the mental deficiencies suffered by DM1 subjects.

P0863. Functional Consequence of Two Novel Dominant Mutations in the Muscle Chloride Channel Gene *CLCN1* Causing Thomsen's Syndrome

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Autosomal dominant myotonia congenita - Thomsen's disease - and autosomal recessive myotonia congenita - Becker's disease - are rare mostly nondystrophic disorders both due to mutations in the *CLCN1* gene encoding the muscle chloride channel 1. In an attempt to categorize Danish patients with myotonia congenita genetically, we sequenced all 23 exons of the *CLCN1* gene in ten selected patients and identified four novel mutations. Two missense mutations (E193K, M128V) were found in dominant myotonia whereas one missense (T328I) and one nonsense (nt2517ΔCT) mutation were found in recessive myotonia. Apart from the novel mutations we also found the previously described mutations; P480L, G285E, F307S and ntΔ1437-1450. Surprisingly, the recurrent R894X mutation was found in four pedigrees segregation both in a dominant and a recessive fashion, and the F307S mutation, which has priviously thought to be strictly dominant, was found together with the nt2517ΔCT nonsense mutation, suggesting recessive behavior in this family. Thus, the relation between genotype and phenotype is not straightforward in myotonia congenita. In order to shed light on the genotype - phenotype relation we examined the electrophysiological features of the patients caring the two novel dominant mutations and unexpectedly, found no decrease of the decrement. The functionality of the two mutations was further characterized by whole-cell patch-clamp.

P0864. Cardiac Disorders in BMD Patients with Distal Gene Deletions

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In-frame deletions of distal part of the dystrophin gene are generally associated with classic Becker muscular dystrophy (BMD). Skeletal myopathy has benign course with later presentation and slower progression, but cardiac disorders could show clinical diversity. In this study we analyzed correlation between gene defect and clinical phenotype in a group of BMD patients with dystrophin gene deletions encompassing exons 45-60. Dystrophin gene deletions were detected by standard multiplex PCR method based on simultaneous amplification of deletion prone exons. Clinical evaluation included neurological and detail cardiological examination. The mean time of onset of disease in our patients was 14.4 y. and skeletal myopathy had relatively slow progression rate, so none of them was in severe stage or wheelchair bound. All of the patients were without symptoms of heart failure, but we detected diffrent forms of cardiological disorders, ranged from benign ECG changes to moderate heart function impairment (EF=40%). Cardiac disorders were in correlation with patient age and, to a lesser extent, with muscle dystrophy severity. Herat dysfunction was associated with different types of gene deletions. For example, moderate systolic function impairment had one patient with single exon 45 deletion (age 19 y.) and another patient with deletion of exons 45-47 (age 31 y.).

P0865. Deletion patterns of dystrophin gene and carrier analysis in Hungarian families with Duchenne/Becker muscular dystrophies

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Duchenne muscular dystrophy is an X-linked progressive muscular disorder with an incidence of 1 per 3500 live-born males. Patients become wheelchair-bound at the age of 18-25 years. Becker muscular dystrophy is a less severe allelic form of the disease with an incidence of 1 per 30 000 live-born males.
Deletion pattern analysis of the dystrophin gene was performed in 49 Hungarian patients with Duchenne/Becker muscular dystrophy. The detection of deletions was performed by multiplex PCR technique that enables the simultaneous screening of 18 exons of the dystrophin gene. In 29 cases (59% of total patients), deletions were detected in the most commonly affected exons. With respect to the proximal-distal distribution of the deletions, 82% of the patients had deletions at the 3' end of the gene, 18% of the deletions affected only the 5' end. Distribution pattern in the dystrophin gene deletions showed similarity to that observed in various Western European populations. If deletions were detected in the index patient, identification of female carriers in the affected family was carried out by radioactive Southern blot hybridization using special cDNA probes, a new method in Hungary introduced by our laboratory. In the 15 families examined so far, 46% of female relatives proved to be carriers of the DMD/BMD gene deletions. The cDNA analysis also enables determination of the exact localization and the full size of the deletion in patients. Therefore, the analysis was also performed in 38 patients and additional exon deletions of the dystrophin gene were detected.

P0866. Duchenne/Becker muscular dystrophy-new approach in carrier testing

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Duchenne muscular dystrophy and Becker muscular dystrophy are X-linked recessive neuromuscular diseases caused by mutations in the gene coding for the 427-kD cytoskeletal protein dystrophin. Deletions, or more rarely duplications, of single or multiple exons within the dystrophin gene are responsible for about 65% of DMD or milder, BMD cases. Within the dystrophin gene, these deletions tend to cluster in hot spot. Frameshift deletions result in DMD (with no functional dystrophin protein produced), while deletions that maintain the reading frame produce the BMD phenotype (partially functional dystrophin present). Approximately two-thirds mothers of affected males with known deletions are asymptomatic carrier of DMD and about 30% percent of affected males represent de novo mutations. Current methods used in carrier testing are directed to multiplex PCR and quantitative analysis of products. However these methods are difficult to perform and interpretation can be subjective. In our study we try to develop an effective and exact assay of carrier testing through cDNA. Illegitimate transcription has made possible the analysis of dystrophin mRNA from peripheral blood lymphocytes. Thanks to the fact that deletions are clustered in hot spots we have designed two sets of primers which span the regions of interest. In a case of female carrier two bands should be recognised; one from normal allel and second related to DMD allel. Here we would like to show preliminary data, which in our opinion will be of great benefit in diagnostic laboratory procedure. We believe that in this way any subjective interpretation will be overcome.

P0867. Loss of the chloride channel in DM1 skeletal muscle due to misregulated alternative splicing: a likely cause of myotonia

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Myotonic dystrophy type 1 (DM1) is the most common form of adult onset muscular dystrophy (1 in 8500 individuals). It is a dominantly inherited disorder caused by a CTG trinucleotide expansion in the 3'

untranslated region of the DMPK gene. Nuclear accumulation CUG)n RNA from the expanded allele is proposed to be pathogenic in DM1 by disrupting the function of the splicing regulator, CUG-binding protein (CUG-BP). A predominant feature of DM1 is myotonia, manifested as delayed skeletal muscle relaxation after voluntary contraction. In humans or animal models myotonia can be due to loss of the muscle-specific chloride channel (ClC-1). Here we demonstrate by western blot and RNase protection analysis loss of ClC-1 mRNA and protein in DM1 skeletal muscle. The likely cause is nonsense mediated decay, as premature stop codons are incorporated in the ClC-1 mRNA by aberrant alternative splicing of intron 2 and exons 6b and 7a. We were able to reproduce the DM1 aberrant splicing pattern in normal cells by coexpressing CUG-BP with a ClC-1 intron 2 minigene. We conclude that aberrant regulation of alternative splicing leads to a predominant pathological feature of DM1. We predict that other targets of CUG-BP are misregulated in DM1 patients causing other symptoms of the disease

P0868. A 12-year experience in molecular diagnosis of Duchenne and Becker muscular dystrophies: a comprehensive strategy for mutation detection allows to detect the molecular defect in 90% of the DMD/BMD patients.

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Since 1989, 258 families have been referred to our laboratory for molecular diagnosis of Duchenne (DMD) or Becker (BMD) muscular dystrophies. We have developed a hierarchical mutation screening strategy for mutation identification in the dystrophin gene (Xp21) including the following steps (1) multiplex PCR to detect large intragenic deletions (2) RT-PCR coupled to the protein truncation test (PTT) to scan for rare deletions, duplications, and point mutations, and (3) sequencing, PCR/restriction, DHPLC, or gene dosage analysis (LightCycler, Roche Diagnostics) to confirm point mutations and test for gene dosage alterations at the genomic level. This strategy allows to detect the molecular defect in 90% of the investigated patients. As a result, the families are currently benefiting from accurate carrier-status assessment. Up to now, a total of 71 mutations have been found by the RT-PCR/PTT procedure consisting in 6 exon deletions, 6 duplications, and 59 point mutations (26 nonsense, 17 splice mutations and 16 frameshift). The effects of nucleotide alterations in splice sites were precisely determined by examination of muscle transcripts, and accurate genotype/phenotype correlation was delivered to the clinicians. Further investigations are required to identify the cause of DMD or BMD in the remaining 10% patients in whom the mutation is not identified yet. An alternative mutation scanning method, the Base Excision Sequence Scanning (BESS), is currently being tested in those patients.
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P0869. Insertion of mid-intron cryptic exons in dystrophin mRNA: a novel mechanism of dystrophinopathy

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We describe two cases of Becker Muscular Dystrophy with an aberrant dystrophin transcript containing an unknown sequence precisely intercalated between two intact exons (89 nt between exons 60 and 61 in patient #1; 90 nt between exons 9 and 10 in patient #2). Both insertions introduce a premature stop codon into the transcript. An in silico survey of the now available entire DMD gene sequence showed that these inserts are present in the mid-part of intron 60 (95 kb) and intron 9 (52 kb) respectively, both being flanked by cryptic splice sites. By sequencing each putative cryptic exon in the two patients we found a single substitution (G→T in patient #1; C→T in patient #2), converting a weak donor splice site into a perfect one, corroborating the assumption that the inserted sequences were cryptic exons activated by a point mutation.
Both patients exhibited a BMD phenotype, consistent with the coexistence of the aberrant transcript with a normally spliced transcript and a weak normal sized dystrophin. Patient #1 was severely mentally retarded.
The activation of cryptic exons by a point mutation is not a novel mechanism, but to our knowledge it has not been reported so far in the recently deciphered gigantic DMD gene introns. This mechanism seems to be unfrequent since we found only 2 such cases in our collection of 720 DMD/BMD patients with a documented mutation. We emphasize that this type of mutation, now explorable, cannot be directly detected at the genomic level without prior transcript analysis.

P0870. Sequencing Of The 79 Exons Of The Dystrophin Gene In Duchenne And Becker Muscular Dystrophies: Identification Of 45 Point Mutations.

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In 1996, we took over molecular diagnosis of dystrophinopathies from the Rhône-Alpes region of France, previously performed in two distinct laboratories. 1816 DNA from 326 families are progressively reanalyzed. In 161 studied families, 81 deletions were identified by multiplex-PCR 18 exons (50%).
In the absence of deletion, our strategy to explore the gene depends on the feasibility of a muscle biopsy. When muscular tissue is available, sequencing of cDNA is used to seek for mutations. 5 point mutations, 1 deletion and 1 duplication were identified this way.
If the biopsy is impossible, strategic choice becomes delicate. As the gene is large, sequencing of all exons doesn't seem the appropriate one at first. It is though the approach we chose for different reasons: 1) we have in our laboratory an old version of sequencer which is not very suitable for screening techniques, 2) the patients being hemizygotes, one sequence is sufficient to explore an exon. A little more than 2 gels are necessary to sequence a patient's all exons, 3) samples were usually collected long ago and the patients are not approachable for biopsy.
Some exons are co-amplified, reducing the number of PCRs to perform (69 vs 79).
77 patients were sequenced: 69 on genomic DNA, 8 on cDNA, allowing 73% detection: 5 deletions outside the hot-spots, 1 duplication and 50 point mutations (20 non-sense, 16 splicing, 14 frameshift mutations). Among the 21 patients without mutation, only 7 were completely sequenced. They probably carry undetected duplications or intronic mutations.

P0871. Familial Hypertophic Cardiomyopathy: many genes, how many diseases?

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Many genetic conditions are considered a single disease. However, molecular analysis often revealed a wide genetic heterogeneity. Recently, new classifications based on the molecular defect, rather than clinical presentation, have been proposed.
Familial Hypertrophic Cardiomyopathy (FHC) is transmitted as autosomal dominant trait with a prevalence of about 1/500. The disease is characterised by a hypertrophied and non-dilated left ventricle. The clinical course of the disease is heterogeneous: some patients remain asymptomatic, others die suddenly. Mutations causing disease in ten cardiac contractile proteins have been identified in FHC patients. Recently mutations on a non sarcomeric protein gene have also been identified as responsible of FHC.

Genotype-phenotype correlation is crucial to the understanding of the natural history of FHC and possibly to separate heterogeneous clinical presentations into different diseases. Genetic definition of FHC may also have to be reconsidered including the clinical interpretation of possible recessive mutations, double heterozygous mutations, and mutations on two genes in the same subject.
We believe that it is crucial to perform the molecular characterisation of patients on several loci. Due to the large number of genes responsible for this disease, we have started a pilot study to organise an Italian laboratory diagnostic network, and we look forward to join other European laboratories working in the same field.
Our activity has focused on search of mutations in MYH7, MYBPC3, TPM1 and TNNT2 genes using the DHPLC technology and automated sequencing. A total of 26 different mutations have been identified. Specific cases will be reported in our presentation.

P0872. Various forms of worldwide quadriceps sparing myopathy are caused by mutations in the UDP-N-acetylglucosamine 2-epimerase/ N-acetylmannosamine kinase gene

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Hereditary Inclusion Body Myopathy (HIBM) (MIM600737) is a unique group of neuromuscular disorders characterized by adult onset, slowly progressive distal and proximal weakness and a typical muscle pathology including rimmed vacuoles and filamentous inclusions. The autosomal recessive prototype form described in Jews of Persian descent and later of other Middle Eastern origins (Iraq, Afghanistan, Kurdistan, Uzbekistan, Egypt) affects mainly leg muscles but with an unusual distribution that spares the quadriceps. We have identified the gene encoding for UDP-N-acetylglucosamine2-epimerase/N-acetylmannosamine kinase (GNE), at chromosome 9p12, as the disease causing gene in this community, where a single homozygous missense mutation (Met712Thr) has been found. Further study of the involvement of GNE in HIBM affected families from various ethnic origins identified ten novel mutations: an homozygous missense mutation in a consanguineous family from Mexico and distinct compound heterozygotes in HIBM-quadriceps sparing non Jewish families from Germany, The Bahamas, Italy, Georgia (USA), and in a large family from East India. Furthermore, interestingly, the GNE "Persian mutation" was also found in HIBM atypical patients with unusual muscle weakness distribution (quadriceps involvement, unusual proximal leg involvement, mild facial weakness) and with unusual occurrence of inflammation, known to appear only in the sporadic form of inclusion body myositis (IBM).
The identification of GNE as the responsible gene for HIBM allows not only the re-evaluation of the phenotypic and genotypic scope of multiple worldwide recessive HIBM forms, but also its involvement in the sporadic form of the disease which is the most common myopathy in individuals over age fifty.

P0873. Familial and sporadic forms of central core disease are associated with mutations in the C-terminal domain of the skeletal muscle ryanodine receptor

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Central core disease (CCD) is an autosomal dominant congenital myopathy. Diagnosis is based on the presence of cores in skeletal muscles. CCD has been linked to the gene encoding the ryanodine receptor (RYR1) and is considered as an allelic disease of Malignant Hyperthermia Susceptibility. However, the report of a recessive form of transmission together with a variable clinical presentation has raised the question of the genetic heterogeneity of the disease. Analyzing a panel of 34 families exclusively recruited on the basis of both clinically and morphologically expressed CCD, 12 different mutations of the C terminal domain of RYR1 have been identified in 16 unrelated families. Morphological analysis of the patients' muscles showed different aspects of cores, all of them being associated with mutations in the C terminal region of RYR1. Furthermore, we characterized the presence of neomutations in the *RyR1* gene in four families. This indicates that neomutations into the *RyR1* gene are not a rare event and must be taken in account for genetic studies of families that present with congenital myopathies type "Central Core Disease". Three mutations led to the deletion in frame of amino acids. This is the first report of amino-acid deletions in RYR1 associated with CCD. According to a 4- transmembrane domains model, the mutations concentrated mostly in the myoplasmic and luminal loops linking respectively transmembrane domains T1 and T2 or T3 and T4 of RYR1.

P0874. Results of mutation analysis in candidate genes for Emery-Dreifuss muscular dystrophy

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So far two disease genes, STA and LMNA, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Screening of patients with EDMD revealed, that mutations in these two genes together account for only 45 % of the cases. Obviously, further genes are likely to be involved in EDMD. Unfortunately, most patients are sporadic cases and families for a positional cloning approach are rare. Thus, we started a candidate gene approach. Emerin encoded by STA and lamin A/C encoded by LMNA are components of the inner nuclear membrane and the nuclear lamina. Thus it seems very likely, that other genes encoding functionally related proteins can cause this disease. Additionally, we considered such genes as candidates, which are specifically expressed in heart and skeletal muscle, that represent the mainly affected tissues in EDMD. Thus, 118 EDMD patients, that were excluded to have mutations in STA and LMNA, were included in the candidate gene mutational analysis. Using genomic DNA of the patients, the exons including the intron/exon boundaries and the promoter regions of candidate genes were amplified by PCR and screened by heteroduplex analysis combined with direct sequencing. Currently, 12 genes (BAF, DDX16, FLNC, LAP1, LAP2, LBR, LMNB1, LMNB2, MAN1, NRM, PSME3, SMPX) are under investigation. So far no disease causing mutation has been found. However, 17 intragenic SNPs have been identified including one in the translated region of FLNC leading to an amino acid exchange. Further upcoming genes related to the nuclear membrane and lamina have to be involved to complete the study.

P0875. First description of mild phenotypes of Ullrich congenital muscular dystrophy caused by mutations in *COL6A3.*

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Ullrich Congenital Muscular Dystrophy (UCMD) is an autosomal recessive disorder characterized by generalized muscular weakness, contractures of multiple joints and distal hyperextensibility. Homozygous mutations of *COL6A2* on chromosome 21q22 have recently been shown to cause UCMD. We performed a genome-wide screening with microsatellite markers in a consanguineous family with three UCMD affected sibs. Linkage of the disease to chromosome 2q37 was found in this family and others. Analysis of *COL6A3,* which encodes the alpha3 chain of collagen VI, led to the identification of a homozygous mutation in three families.
A nonsense mutation, R2342X, caused absence of collagen VI in muscle and fibroblasts and a severe phenotype, as described in UCMD patients. A splice site mutation (6930+5A>G), leading to the skipping of an exon, caused a partial reduction of collagen VI in muscle biopsy and an intermediate phenotype. A nonsense mutation R465X was associated with only a limited reduction of collagen type VI around patient muscle fibers. This was due to nonsense mediated exon skipping and could explain the mild phenotype of the patient who was ambulant at the age of 18 years and showed an unusual

combination of hyperlaxity and finger contractures.
Mutations in *COL6A3* are described in UCMD for the first time, and illustrate the wide spectrum of phenotypes which can be caused by collagen VI deficiency.

P0876. Facioscapulohumeral muscular dystrophy in Romania

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Facioscapulohumeral muscular dystrophy (FSHMD) is characterized by a considerable variability in terms of the severity of symptoms, onset age and changes at muscular level. FSHMD can be clinically distinguished from the other progressive muscular dystrophies by: changes in the face appearance (tapir lip) and scapulohumeral girdle, as well as slow evolution. FSHMD is a myopathy with autosomal dominant inheritance and incomplete gene penetrance. The locus of FSHMD gene maps to 4q35-3 ter. The studies were performed on 180 FSHMD cases. The analysis of the pedigrees of the patients investigated confirms the autosomal dominant mode of inheritance. The analysis of FSHMD onset age in patients from the same family (ancestry and descent) shows the presence of the anticipation phenomenon (earlier age at onset in successive generations). Incomplete FSHMD gene penetrance is demonstrated by the intrafamilial variability of the severity and evolution of the disease: from almost asymptomatic patients to wheelchair dependent patients. Our study found a slight prevalence of the disease in the male sex (52.68%) compared to the female sex (47.32%). Epidemiological studies have found a higher FSHMD incidence in Brasov, Constanta, Ilfov, Prahova, Salaj and Sibiu districts, which can be explained by the effect of the founder couple and genetic drift. The study of the incidence and clinical genetic aspects of this form of myopathy which represents 9% of all PMD types and generates serious socio-economic problems (being a disabling disease) is motivated by the necessity of offering efficient genetic counseling to FSHMD patients and their families.

P0877. Molecular and Clinical Studies of Facioscapulohumeral Muscular Dystrophy (FSHMD) in Greece

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FSHMD is a myopathy transmitted by autosomal dominant inheritance.The genetic locus has been mapped to the 4q35 subtelomeric region. The telomeric probe p13E-11 has been shown to detect EcoRI polymorphic fragments shorter than 35kb and an EcoRI-BlnI (or AvrII) digestion is used to avoid the interference of small EcoRI polymorphic fragments of 10qter
origin. We studied 45 Greek families, 59 affected and 21 unaffected individuals at risk of inheriting or transmitting the FSHMD shorter frgments. Restriction analysis of genomic DNA using EcoRI and EcoRI/AvrII enzymes, followed by pulse-field or conventional gel electrophoresis and non radioactive hybridization with p13E-11 probe, were performed. The results revealed an EcoRI/AvrII fragment, ranging between 7.5 and 34kb, in 32 families (74%), comprising 19 familial and 13 isolated cases. In all, except one, FSHMD familial cases the same size fragment segregated in the family. In two isolated cases, the presence of three shorter fragments, complicated the interpretation of Southern blot analysis. An overall correlation has been found, between fragment size, age of onset and disease severity, indicating that patients with EcoRI/AvrII fragment smaller than 20kb are more severely affected than patients with larger fragments. The application of double digestion, identifies FSHMD alleles even in pre-symptomatic cases, facilitates clinical prognosis and allows genetic counselling of the disease.

P0878. Mutations in the Selenoprotein N gene (SEPN1) cause congenital muscular dystrophy with early rigidity of the spine and restrictive respiratory syndrome

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Rigid Spine Muscular Dystrophy (RSMD) is a rare autosomal recessive neuromuscular disorder characterized by early rigidity of the spine, axial and proximal muscle weakness associated with a dystrophic pattern of patient muscle biopsies, limb-joint contractures, and restrictive respiratory insufficiency requiring nocturnal ventilation. We recently reported the refinement of the RSMD1 locus on 1p35-36 to a 1cM region by linkage disequilibrium and the identification of mutations in SEPN1, the gene encoding a recently described selenoprotein of unknown function, selenoprotein N. Selenoproteins have in common to contain selenium as selenocysteine. One of the unique features in the incorporation of selenocysteine is the use of a UGA codon, which normally serves as a termination signal and needs a mRNA stemloop structure located in the 3' unstranslated region and specific translation factors to be recognized as the codon for selenocysteine insertion. Fourteen different mutations including frameshift, missense, nonsense mutations in the coding sequence and a splice-site mutation, have been identified in SEPN1. Previous Northern blot experiments showed an ubiquitous expression of SEPN1. Polyclonal antibodies were developed in order to perform additional studies at the protein level. Biochemical studies with these antibodies allowed the detection of a 70 kDa band corresponding to the full-length protein present in control fibroblasts or myoblasts. However, this band could not be detected in total proteins extracted from patients cells bearing a homozygous frameshift mutation. The cellular localization of the selenoprotein N is currently underway and might help to better understand the role of this protein in skeletal muscle.

P0879. Conversion analysis between SMN 1 and SMN 2 genes in patients with a spinal muscular atrophy from North-West region of Russia.

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Spinal muscular atrophy (SMA) is the second most common fatal autosomal recessive disease with the frequency 1: 10 000 newborn. The results of deletion and conversion analysis in 53 SMA families from North-West region of Russia are reported. Homozygous deletions of SMN 1 gene were identified in 96% of our patients. 21% of our SMA patients had the absence of exon 7 SMN1 gene but retention of exon 8. This complex rearrangements might result either from extensive deletion (up to exon 8) or from gene-conversion event giving rise to the origin of "chimeric" gene. The latter has been registered in 13% of SMA patients. All our conversion cases could be attributed to the formation of "chimeric" genes consisting of 5' area of SMN 2 gene (exon 7) and 3' area of SMN 1 gene (exon 8). Thus two types of conversion chromosomes were identified: with the deletion of exon 7 of SMN 2 gene as result of "chimeric" gene formation (1) and without such a deletion (2).

P0880. SMN mutations screening in non deleted SMA patients

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Spinal muscular atrophy (SMA) is linked to 5q13 locus in 95% patients. Among them, 98% show homozygous deletion of SMN1 gene, while about 2% of them show heterozygous deletion of SMN1 associating a mutation on the only SMN1 copy they present, and thus required a specific approach to reach diagnosis.
Here we show the results of SMN1 analysis in this specific group of SMA patients among those referred for molecular diagnosis to our laboratory. Genotype was established in all patients with clinical course and electromyography consistent with spinal muscular atrophy diagnosis and not showing homozygous SMN1 deletion. Detection of patients carrying a heterozygous SMN1 deletion was performed

according to the fluorescent quantitative assay described by Gerard et al. (2000). Three patients carrying this genotype were identified and screened for SMN1 mutation with a standardized method associating single strand conformational polymorphism analysis and long range PCR to demonstrate that the detected mutations are indeed localized on SMN1 not SMN2, his homologous gene. Using this strategy, we successfully identified the causative mutations in all 3 studied patients. Two novel mutations were described in exon 3 and the third one was the previously described Y272C in exon 6.

P0881. Molecular genetic study of spinal muscular atrophy in Russia.

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Spinal muscular atrophy is a common often lethal neurodegenerative disorder with three major clinical phenotypes (SMA type I, II, III). The disease is caused by deletion, conversion or point mutations in the telomeric survival motor neuron gene (SMNt). In our study, we present the molecular-genetic analysis of 372 patients from 369 Russian SMA families. Homozygous deletions of either one exon 7 or both exons 7 and 8 of SMNt have been demonstrated in 96,2% of our patients (99%, 96% and 90% for SMA I, II and III respectively). The absence of SMNt exon 7 but retention of exon 8 were revealed in 59 (16 %) SMA patients. “Chimeric” (SMNc-SMNt) gene was found in 9% of SMA I, in 16% of SMA II and in 15% of SMA III patients. SMNt/SMNc ratio was analyzed by densitometry analysis (Gel Doc 2000/ Bio-RAD) of 7 and 8 exons PCR products digested by Eco RV and Bse NI respectively. Twenty families were referred for prenatal diagnostics without accessible material from affected child. Parents in sixteen families were heterozygous carriers of SMNt deletion. Prenatal diagnostics has been performed in 118 SMA families by means of deletion analysis of SMNt gene and polymorphic DNA-markers (D5S435, D5S557 and D5S629). We identified 31 affected, 58 carrier and 29 normal individuals among observed fetuses.

P0882. Catalytic nucleic acids for specific inhibition of SMN gene expression

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease caused by loss of functional *survival motor neuron* gene (*SMN*) product. SMA ultimately leads to progressive loss of motor neuron function and muscular atrophy. Although the *SMN* gene is ubiquitously expressed, the cause for selective motor neuron loss is unknown. Study of the disease has been hampered by the fact that the condition is embryonal lethal for mice, and other currently available transgenic mice models are not viable for long periods of time. Goal of this study is to develop a cell culture system in which the *SMN* gene expression can be varied using catalytic nucleic acids. Catalytic nucleic acids are short sequences of RNA (ribozymes) or DNA (DNAzymes) capable of sequence specific cleavage of a target mRNA, thus downregulating gene expression. We designed three ribozymes and three DNAzymes targeted against the murine *Smn* RNA sequence. All ribozymes and DNAzymes effectively cleaved the full length *Smn* RNA in a sequence specific manner, while inactive versions of the molecules had no effect. Cleavage of target RNA was observed at magnesium concentrations as low as 2 mM, which corresponds to the intracellular Magnesium concentration of mammalian cells. Cleavage increased in a time and concentration dependent manner. These results indicate that catalytic nucleic acids effectively cleave *Smn* target RNA in a cellular environment and thus have great potential for interference with *Smn* gene expression in cells and *in vivo*.

P0883. Hyperacetylating agents activate SMN2 gene expression in fibroblast cultures from spinal muscular atrophy (SMA) patients

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder caused by homozygous loss of the Survival of Motor Neuron (SMN1) gene. SMN1 is located in a large inverted duplicated region on 5q13 where an almost identical copy, SMN2, is also present. The two genes differ only for a silent mutation in the reading frame, resulting in the majority of SMN2 transcripts lacking exon 7. All patients retain at least one, more often two to four, copies of SMN2. Milder phenotypes are generally associated with higher gene copy number and higher levels of protein, although patients with different phenotype can carry the same number of SMN2 copies. These data suggest that SMN2 genes are functionally different and one possible mechanism responsible of such differences could be epigenetic modifications, like DNA methylation and/or histone acetylation. Thus, upregulation of SMN2 by chromatin remodelling agents can be a potential target for a therapeutic approach to SMA.
We have currently undertaken a study to evaluate the effect of hyperacetylating agents, such as sodium butyrate and derivatives, on SMN2 expression in primary fibroblast cultures from patients with different disease severity. Preliminary results show that the treatment significantly increases the production of full length SMN2 transcripts and the number of gems (the nuclear structures where the SMN protein concentrates). These encouraging results suggest that hyperacetylating agents can be beneficial in SMA treatment protocols.

P0884. Two approaches to therapy for Muscular Dystrophies in Russia.

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Two approaches in therapy for muscular dystrophies were developed as a part of program for the long-term support for families with hereditary neuromuscular disorders (HNMD) in which included clinical trials of medicaments treatment (prednisolone, cyclosporine) and myoblast transplantation for DMD gene correction; the creation of computer database of Russian families with HNMD; the use of common diagnostic criteria with DNA-analysis et cetera.
94 DMD/BMD and 10 LGMD patients-volunteers participated in double-blind controlled prednisolone trial during 1 year with alternate-day schedule (0.5 mg/kg/day in treatment day). In 80% cases were obtained some beneficial effects and in all cases were absent the manifested side-effects. 17 DMD/BMD patients obtained this treatment 3-6 years and maintained relatively good conditions with low progression of muscular weakness.
For the other approach it was developed special technique of preparation of human myoblast cultures. 5 DMD patients-volunteers participated in clinical trial of myoblast transplantation by protocol “single muscle treatment”. Every recipient received cyclosporine two weeks before transplantation and one month after transplantation. 50-90 millions of myoblast cells was transplanted into m. tibialis anterior of one leg. 6 months after transplantation in biopsy specimens were revealed the presence of donor’s DNA (in three cases) and the expression of dystrophin (in two cases). Dystrophin and donor’s DNA were absent in the sham-injected muscles.

P 19. Neurogenetics

P0885. A first locus for common simple Febrile seizures

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We report a large multigenerational French family with a homogeneous phenotype consisting of isolated simple Febrile Seizures (FS). The FS trait did not show any linkage with the reported loci for FS and Generalised Epilepsy with Febrile Seizures (GEFS+). After a genome scan, a new locus for FS was identified.
Patients and methods: Clinical study: Our family consisted of 166 individuals on 5 generations. Affected members presented a history of simple FS that segregates as autosomosal dominant trait. Genotyping and linkage analysis: After exclusion of reported loci for FS and GEFS+, a genome-wide search was performed with 380 markers.
Results: All affected members presented FS that fulfil the criteria

of simple FS. All FS ceased before 5 years of age and no later afebrile seizures or epilepsy were reported. In the oldest generation (II), the status of all members was considered as unknown for the reliability of the genetic analysis. The genome wide search allowed the identification of a new candidate region (Zmax=3.31 at q=0.00). Multipoint analysis and haplotypes construction confirmed the genetic linkage of this region to simple FS trait.
Conclusion: Our family presents a homogeneous phenotype consisting of isolated simple FS, the commonest form of FS. A new locus is identified in this family and the sequence analysis of a potential candidate gene is in progress.

P0886. Clinical and Genetic Analysis of a New Multigenerational Pedigree with GEFS+ (Generalized Epilepsy with Febrile Seizures Plus)

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Febrile seizures (FS) affect 3% of all children under six years of age. A small proportion of children with FS later develops epilepsy. While most FS show complex inheritance, a small proportion of FS is autosomal dominant. Two FS genes have been localized at 8q14-q21 and 19p. The syndrome of generalized epilepsy with febrile seizures plus (GEFS+) is a heterogeneous disorder characterized by febrile seizures that may persist beyond the age of six, and non-febrile seizures. GEFS+ is an autosomal dominant disorder and three genes (SCN1A, SCN1B, GABRG2) have been identified to date at 2q34, 19q13, and 5q34, respectively, while a fourth GEFS+ locus (5q14-q15) has been suggested.
A large multigenerational GEFS+ family was collected in France. All affected members had typical FS. Among them, seven had other types of seizures including FS after the age of 6 years, non-febrile generalized seizures, or partial seizures later in life.
Exclusion study of candidate genes and loci was performed with penetrance at 0.9 and phenocopy rates at 0.02 or 0.03. Multipoint LOD scores < -2 were obtained at 5q14-q15, 8q14-q21 and 19p. In addition, the genomic areas surrounding the SCN1A, SCN1B and GABRG2, were also excluded.
Our data provide further evidence for the high level of genetic heterogeneity associated with familial febrile seizures and GEFS+, and prove the existence of a new GEFS+ gene situated elsewhere in the genome. Genome-wide scan is now underway in order to localize this new GEFS+ gene and preliminary data will be presented.

P0887. A novel KCNA1 mutation in a large family with Episodic Ataxia type 1

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Familial episodic ataxia are rare genetically heterogeneous neurological disorders. Type 1, or myokymia with periodic ataxia (OMIM 160120) is characterized by short-lasting attacks of ataxia, sometimes accompanied by jerking movements, episodes of dizziness and permanent tremor of the head and hands (myokymia). This disorder has been ascribed to alteration of a potassium channel subunit, encoded by the KCNA1 gene, localized in 12p13.
We describe a large French family with 11 affected patients over 3 generations. The propositus, a young lady, is particularly handicapped by myokymia. She was previously diagnosed as having a psychiatric disturbance.
Direct DNA sequencing of the KCNA1 gene disclosed a novel heterozygous misense mutation, leading to a Leucine to Phenylalanine substitution at position 305, in the 4th transmembrane domain. The mutation was confirmed by enzymatic restriction analysis with the disappearance of an AluI cutting site. This Leucine residue is highly conserved through evolution, from drosophila to human. All the studied affected family members carried this mutation that is absent in 100 unrelated and unaffected individuals. Therefore, it is likely that this Leu to Phe substitution at position 305 is pathogenic. Only speculations can be made regarding the possible consequences of the Leu to Phe substitution for the K+ channel function.
This autosomal dominant disorder is important to recognize, not only to avoid a erroneous diagnosis of psychiatric disturbance that frequently mislabel these patients, but also to offer specific therapies.

P0888. Cognitive impairment in autosomic dominant pure spastic paraparesis SPG4

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Sixty five individuals from 10 French families with identified mutations of the spastin gene (SPG4) participated in a study on cognitive function. The study was conducted to verify previous reports on the association of cognitive impairment and hereditary pure spastic paraparesis. Thirty six carriers (24 female, 12 male) including 5 asymptomatic at the time of the study as well as 29 of their non-carriers siblings (18 female, 11 male) were included in the study. The Cambridge Cognitive Examination test (CAMCOG) was used in all subjects, supplemented by additional neuropsychological tests for 30 individuals to evaluate executive functions, memory and visual form discrimination.
There was no overall significant difference between carriers and non-carriers. However, in the group with age over 50 years, a significant cognitive impairment was found in carriers compared to non-carriers, with lower scores in several parameters of executive functions. Similar results have been reported previously in Irish families with spastic paraparesis. These findings suggest a wider involvement of subcortical and, may be, cortical functions even in pure spastic paraparesis after 50 years.

P0889. Iron-binding properties of frataxin and its homologues

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Friedreich's ataxia (FRDA), an autosomal recessive cardio- and neurodegenerative disease, is caused by low expression of frataxin: a small mitochondrial protein, encoded into the nucleus. At biochemical level the lack of frataxin leads to disregulation of mitochondrial iron homeostasis and oxidative damage which eventually causes neuronal death. Recently, it has been reported the Yfh1 (yeast frataxin homologue) shows a ferritin-like behaviour in vitro: the protein was shown to form large aggregates able to sequester iron form solution in the presence of an iron excess (1). No direct iron binding was however shown for human frataxin under similar conditions (2). We have carried out an exhaustive study on three frataxin orthologues from E. coli, yeast and from human with the aim of further testing the working hypothesis of a direct involvement of frataxin in iron binding. Using
these three proteins, selected as representatives of different evolution steps, we have characterised their fold and thermodynamic stability, compared their ion binding specificity and their tendencies to aggregate. A number of mutants was produced to identify the protein surface involved in these functions. Our work leads us to a more complex and complete picture of the binding and aggregative properties of frataxins.
References
1) Adamec J, Rusnak F, Owen WG, Naylor S, Benson LM, Gacy AM,Isaya G.
Am J Hum Genet. 2000 Sep;67(3):549-62.
2) Musco G, Stier G, Kolmerer B, Adinolfi S, Martin S, Frenkiel T, Gibson T, Pastore A
Structure Fold Des. 2000 Jul 15;8(7):695-707.

P0890. Spastin, the protein mutated in autosomal dominant hereditary spastic paraplegia, is involved in microtubule dynamics

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Hereditary spastic paraplegia (HSP) is a neurodegenerative disease characterised by weakness and spasticity of the lower limbs due to degeneration of the corticospinal tracts. The gene responsible for the most frequent form of autosomal dominant HSP (SPG4) encodes spastin, an ATPase belonging to the AAA proteins family. Interestingly, almost all the missense mutations found in HSP patients fall into the AAA functional domain.
The cellular pathways in which spastin operates and its role in

causing degeneration of motor axons are still unclear. By expressing wild-type or ATPase-defective spastin in several cell types, we show that spastin interacts dynamically with microtubules. This association is mediated by the N-terminal region of the protein and regulated through the ATPase activity of the AAA domain. Expression of missense mutations falling into the AAA cassette leads to constitutive binding to microtubules in transfected cells and induces the disappearance of the aster and the formation of thick perinuclear bundles, suggesting a role of spastin in microtubule dynamics. Moreover, overexpression of wild-type spastin seems to promote microtubule disassembly in transfected cells. These data suggest that the degeneration of corticospinal axons, in HSP patients, could be due to impairment of fine regulation of the microtubule cytoskeleton. To better understand spastin localization, specific antibodies have been produced and experiments to determine the endogenous protein localization are in progress. Furthermore, a recombinant spastin obtained with the baculovirus system will be used in microtubule severing assay in order to confirm the hypothesized role of spastin in microtubule disassembly.

P0891. Mice lacking paraplegin, a mitochondrial AAA protease involved in hereditary spastic paraplegia, show axonal degeneration and abnormal mitochondria

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Hereditary spastic paraplegia (HSP) is a progressive neurological disorder characterized by degeneration of the corticospinal tracts. The gene responsible for the autosomal recessive form linked to chromosome 16q (SPG7) encodes paraplegin, a mitochondrial ATPase involved in protein quality control in the inner mitochondrial membrane. In order to study the pathogenesis of HSP due to lack of paraplegin, we have generated a mouse model by inactivation of the Spg7 gene. Paraplegin deficient mice are born at the expected mendelian ratio, are viable, and fertile. At approximately 6 months of age homozygous Spg7 -/- animals start displaying an impaired performance on the rotarod apparatus. Semithin sections of the spinal cord of 7-months-old animals show a variable number of focal axonal swellings in the distal axons of the fasciculus gracilis and of descending spinal tracts, consistent with a retrograde axonopathy. This phenotype is slowly progressive, with signs of axonal degeneration becoming prominent at 12 month of age. Mice older than one year show additional phenotypes, such as axonal swelling and degeneration in the optic and sciatic nerves, and muscle abnormalities. Electron microscopy analysis of affected spinal cords demonstrates that, long before degeneration, axons are filled with mitochondria with abnormal morphology, indicating that mitochondrial dysfunction is likely the cause for the disease. Swollen axons contain accumulated organelles and neurofilaments, suggesting that axonal degeneration may be due to impaired axonal transport. We are currently analyzing whether defective ATP production, oxidative stress or apoptosis are important determinants of pathology in our HSP animal model.

P0892. The transcription factor SOX10 regulates PLP expression in the central nervous system

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SOX10 is an essential factor for the enteric nervous system (ENS), melanocytes and glial cells development. Mutations in the SOX10 gene were described in several cases of Shah-Waardenburg syndrome, a neurocristopathy characterized by the association of Hirschsprung disease (intestinal aganglionosis) and Waardenburg syndrome (pigmentation defects and sensorineural deafness).
In accordance, it was shown that SOX10 controls expression of MITF and RET, which play important roles during melanocytes and ENS development, respectively. Some patients also present with myelination defects of the central (CNS) and peripheral nervous system (PNS), which is in agreement with the demonstration that P0 and Cx32, two major proteins of the PNS, are controlled by SOX10. Nevertheless, these findings cannot explain the defects of the CNS, consistent with Pelizaeus-Merzbacher disease (PMD), observed in one patient. This suggests that SOX10 may regulate other genes involved in the myelination process of the CNS.
To test this hypothesis, we sought the possible involvement of SOX10 in the regulation of expression of PLP and its alternative transcript DM20. Both proteins are major components of myelin in the CNS, and mutations of the PLP gene are associated with PMD. Here we show that SOX10 activates expression of the PLP gene in transfection assays, and that EGR2, another major regulator in the CNS, is also able to regulate the PLP promoter. These results were further confirmed by the study of a cell line expressing SOX10 in an inducible manner, where PLP and DM20 expression is upregulated when SOX10 is induced.

P0893. Inducible mouse models for Friedreich Ataxia

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Friedreich ataxia (FRDA), the most common autosomal recessive ataxia, associates degeneration of the large sensory neurons and spinocerebellar tracts, cardiomyopathy and increased incidence in diabetes. FRDA is caused by severely reduced levels of frataxin, a mitochondrial protein of unknown function. Data from yeast and patients indicate that frataxin defect causes a specific iron-sulfur protein deficiency and mitochondrial iron accumulation, suggesting oxidative damage involvement.
As complete absence of frataxin in mouse was lethal early in development, we have chosen a conditional gene targeting approach, to generate first a heart frataxin-deficient line and a neuron/heart frataxin-deficient line. The heart line reproduces well the different steps of the human cardiac defects, but the neuron/heart line (generated to study the neurological defects) was very severe. Indeed, the mutant mice showed a short life expectancy (25 days), probably due to many additional lesions in brain and other tissues. To better reproduce the natural evolution of the neurological symptoms, we have generated two inducible neuronal frataxin-deficient lines by using tamoxifen inducible recombinase. The deletion of frataxin is induced at 4 weeks of age. In one line the deletion occurs in the central and the peripheral nervous system and the other line presents a deletion more specific of the cerebellum. The mice have a normal life expectancy but develop a progressive spinocerebellar degeneration revealed by histology, electron microscopy and behavioural studies to assess the evolution of the symptoms.
These models represent good models to evaluate treatment strategies for the neurological side of the human disease.

P0894. Huntington's Chorea In The Moscow Region

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Huhtington's chorea is a severe hereditary neurodegenerative disease characterized by choreic hyperkinesis and progressing dementia. The object of the present study was creation of genetic register of the Huntington's chorea in the Moscow Region in order to organize medico-genetic consulting patients using up-to-date diagnostic methods. Direct and indirect registration methods were applied. The total of 111 families suffering from the Huntington's chorea was registered in the Moscow region. Molecular-genetic method was used to confirm the primary diagnosis. Diagnosis was considered confirmed if the number of repeating cytosine-adenine-guanine sequences in the disease gene molecule exceeded 37. Probands' children underwent molecular-genetic investigation to determine disease gene carriers. Siblings were also examined if the disease in question was found in more than one generation. In cases when prenatal diagnosis revealed the disease gene carriage by probands' children, the latter were prescribed to undergo DNA-investigation. Haloperidolum was used for treatment of the Huntington's chorea patients.

P0895. Study of the normal CAG tract at the Huntington disease locus in the Portuguese population

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Huntington disease (HD) is a dominant disorder caused by the

expansion of a (CAG)n localised on the first exon of the gene, which contains (1) 6-26 CAGs in normal stable alleles, (2) 27-35 CAGs in non-pathogenic but expandable alleles, (3) 36-39 CAGs in expanded alleles with reduced penetrance and (4) 40 CAGs in fully-penetrant alleles. Molecular diagnosis is based on the determination of the CAG repeat size by PCR.
Intermediate (class 2) alleles were present in 4.4%, while low-penetrance (class 3) alleles were present in 2.0%, among all 'control' chromosomes (n=249) from our routine genetic testing. This high frequency of unstable alleles led us to study a large control sample (n=1772 chromosomes) from the Portuguese population (50 Guthrie cards from each district in mainland Portugal and the islands of Madeira and Azores).
No differences between normal (classes 1+2) alleles from affected individuals and controls could be shown. Distribution of the (CAG)n size showed: range 9-40 CAGs, mean 18.4±3.2, mode 17, median 17 (skewness 1.3, kurtosis 3). The 17-CAGs allele was by far the most frequent (38.0%). Intermediate alleles (27-35) represented 3.0% in the control population; 2 expanded alleles (36 and 40 repeats, 0.11%) were found. There was no evi-dence for geographical clustering of the intermediate or expanded alleles. This study showed that intermediate alleles at the HD locus are relatively frequent in the Portuguese population, which is particularly important for molecular diagnosis and genetic counselling. This will also be relevant for genetic epidemiology and evolution studies of the HD mutation.

P0896. Common trends in distribution of HLA-DQA1 and DQB1 haplotypes in patients with febrile seizures and epilepsy.

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Objective: The present study was designed to reveal the common trends in distribution of HLA-DQA1 and HLA-DQB1 haplotypes frequencies in patients with febrile seizures (FS) (simple and transformed to afebrile seizures (FST)) and epilepsy to determine the common traits between the pathologies.
Methods: We investigated HLA-DQA1 and HLA-DQB1 haplotypes by RFLP-analysis in the group of 68 children with FS (inclusive 12 patients with FST), 22 patients with epilepsy, and 70 individuals from control group.
Results: Frequency of HLA-DQA1 *0501 haplotype was maximal in control group (0.436), less frequent in patients with FST (0.250), in patients with FS (0.235) and in patients with epilepsy (0.023) with $p < 0.001$ between FS and control group, FST and epilepsy and FST and control group, and $p < 0.1$ between the FST and control group. Frequency of HLA-DQA1 *0201 haplotype was maximal in patients with epilepsy (0.318), less frequent in patients with FST (0.125), in patients with FS (0.081) and in control group (0.043), with $p < 0.001$ between epilepsy and control group. Frequency of HLA-DQB1 *0502 haplotype was maximal in control group (0.093), less frequent in patients with FS (0.088), in patients with FST (0.042) and in patients with epilepsy (0.000) with $p < 0.05$ between patients with epilepsy and control group.
Conclusions: The results suggests common trait in HLA-DQA1 and DQB1 haplotypes distributions in patients with FS, FST and epilepsy and, probably, have a "protective" role.

P0897. Metachromatic leukodystrophy : relations between phenotype and mutations in arylsulfatase A in adult forms.

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Metachromatic leukodystrophy is due to a deficiency in arylsulfatase A which hydrolyses sulfogalactosylceramides and other sulfated glycolipids (sulfatides). In function of age, the clinical manifestations are different. The infantile form is characterized by a regression of acquired motor and later of mental activities. There are also adult forms which do not occur in the same families. Moreover, in the adult, there are two clinical variants, one in which motor signs are predominant, the other in which psychiatric symptoms dominate, although secondarily the patients become bedridden and demented. The evolution in the adult forms may be of several decades. In all those cases in the adult, the enzyme deficiency is identical as well as sulfatiduria which relates to the absence of the catabolic enzyme for sulfated glycolipids. Interestingly, it is well known from the work of V. Gieselmann (reviewed in Human Mut. 4: 233-243, 1994). that the mutations in infantile forms are different from those occurring in the adult which may explain homochrony.There seem to be specific mutations according to the motor and psychocognitive types in the adult, i.e. P426L for motor forms in a homozygote form and in the psychiatric forms a specific I179S mutation as a compound heterozygote.Studies are in progress to determine the precise clinical characteristics of the psychiatric forms and whether I179S mutation of arylsulfatase A could be a susceptibility factor of schizophrenia.

P0898. Correction of the biochemical phenotype in an X-Linked adrenoleukodystrophy mouse model by transgenic overexpression of the ALDR gene: functional redundancy at the peroxisomal membrane?

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X-linked adrenoleukodystrophy is a neurological disorder presenting with central or peripheral demyelination and impaired function of adrenals. X-ALD patients accumulate very long chain fatty acids (VLCFA) in plasma and tissues, mainly adrenal cortex and nervous system. The two main neurological phenotypes are the severe childhood cerebral form and the slowly progressive adult adrenomyeloneuropathy. A mouse model of the disease also accumulates VLCFAs in target organs, and has recently been shown to develop an adrenomyeloneuropathy-like phenotype (Pujol et al, Hum Mol Genet in press).
The gene mutated in the disease codes for a peroxisomal ABC transporter protein (ALDP). There is other three peroxisomal ABC transporters, ALDRP being the closest homolog (88% similarity with ALDP at the aminoacid level). A working hypothesis is that ALDRP could play a similar biochemical function. To assess whether an overexpression of ALDRP in target organs could compensate the biochemical phenotype of the ALD deficient mouse, transgenic mice overexpressing ALDRP in target organs were generated (ALDRtg), and crossed to ALD KO mice (ALD KO/ALDRtg).
We have compared the ALD KO and ALD KO/ ALDRtg have found that overexpression of the ALDRP leads to full correction of C26:0 and C24:0 fatty acids levels and of the ratios C26:0/C22:0 and C24:0/C22:0 in adrenal gland and CNS. Preliminary results indicate a correction of histopathology in adrenals. It remains to be seen whether this normalisation of the biochemical phenotype leads to an amelioration of the neurological AMN-like phenotype in mice.

P0899. New families with ataxia and hearing and visual impairment (van Bogaert-Martin syndrome) linked to 6p21-23 and refined genetic localisation

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The hereditary ataxias are a heterogeneous group of genetic disorders characterised by cerebellar symptoms associated with others neurological and non neurological features. Van Bogaert-Martin syndrome (MIM#271250) is defined by childhood onset autosomal recessive ataxia with optic and cochlear degeneration leading to blindness and deafness. We have previously reported that this condition is linked to a 17 cM region in 6p21-23 in an Israeli family.
We have analysed a set of patients born from consanguineous parents and for whom a diagnosis of Friedreich ataxia, ataxia with vitamin E deficiency or ataxia linked to 9q34 (with elevated alpha-foetoprotein) was excluded. Four patients, from three families were homozygous over part of the 6p21-23 interval. A sister and a brother of Turkish origin were haploidentical in 6p21-23 and homozygous over 6 consecutive markers defining an 11 cM interval. They developed ataxia and a peripheral demyelinating neuropathy before age 10, but had no hearing impairment. The sister developed retinitis

pigmentosa by age 15, which was not present in the younger brother. The two other patients, of Israeli and Lebanese origin respectively, were homozygous over at least 6 consecutive markers. Albeit it cannot be formally excluded that one or both are homozygous by chance (due solely to consanguinity and not by linkage), the minimal region of homozygosity would suggest that the defective gene is located in a 7 cM interval located between markers D6S1660 and D6S265.

P0900. Further evidence that SPG3A gene mutations cause autosomal dominant hereditary spastic paraplegia

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Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a genetically heterogeneous neurodegenerative disorder characterized by progressive spasticity of the lower limbs.
In the past few years, eight spastic gait (SPG) loci have been shown to be associated with the "pure" form of AD-HSP, but only one gene responsible for the SPG4 locus has been identified.
Very recently, the gene responsible for the SPG3A locus was also identified.
The coding sequence is divided into 14 exons spanning approximately 69 Kb. The peptide encoded by SPG3A, atlastin, shows significant homology with several GTPases. Atlastin contains three conserved motifs: P-loop (74GAFRKGKS81); DxxG (146DTQG); and RD (217RD) that characterize guanylate binding/GTPase active sites.
We report a novel mutation in a large Italian pedigree from southern Italy with AD-HSP. Significant linkage to the SPG3 locus on chromosome 14 was detected with a maximum LOD score of 4.58 at D14S255.
Direct sequencing of the SPG3A gene revealed a G→A mutation at position 818 in the exon 7 of the gene. This mutation created an amino-acid change from Arg to Gln at codon 217. The variation was also confirmed with restriction enzyme Taq I, which cleaved the wild-type PCR product of 208 bp into 145 bp and 63 bp digestion fragments, but did not cleave the corresponding region of the SPG3A gene in our patients. Complete co-segregation of the heterozygous mutation with the disease was observed. This mutation was absent in 100 control chromosomes examined.
These data confirm that mutations in the SPG3A gene are causative of AD-HSP.

P0901. Linkage analysis in an italian family affected by autosomal dominant pure hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) includes a heterogeneous group of neurodegenerative disorders characterised by progressive spasticity of the lower limbs. Clinically, HSP has been divided in pure and complicated forms. In pure HSP no other neurological features are present and slowly progressive spastic gait is usually associated with mild decrease of vibration sense and sphincter disturbances. The mode of inheritance may be autosomal dominant, autosomal recessive or X-linked. So far, seven loci responsible for autosomal dominant pure HSP (ADPHSP) have been mapped to chromosomes 14q (SPG3), 2p (SPG4), 15q (SPG6), 8q (SPG8), 12q (SPG10), 19q (SPG12) and 2q (SPG13). Two ADPHSP genes have been identified so far, the SPG4 gene (Spastin) and the SPG3 gene (Atlastin).
Here we report an Italian family with 10 individuals affected by ADPHSP spanning three consecutive generations. The way of inheritance is autosomal dominant, with high penetrance.
The phenotype is characterised by a high incidence of urinary disturbances, mild muscle weakness and wasting, and benign course (only two patients were wheelchair-bound after 10 to 20 years of disease). Patients often complained of mild lower limbs paresthesias and diurnal fluctuations of spasticity. Mutations in the Spastin gene were excluded by direct sequencing of all 17 exons of the gene. Linkage with all known ADPHSP loci was also excluded. A genome wide search using 400 microsatellite markers covering all autosomes allowed to map a novel ADPHSP locus, termed SPG18.

P0902. Linkage analysis in an Italian family with autosomal recessive hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders of the motor system, characterized clinically by slowly progressive lower extremity spasticity and weakness, in which dominant, recessive and X-linked forms have been described. While autosomal dominant HSP has been extensively studied, autosomal recessive HSP is less well known and it is considered a rare form. Until now, five families (four Tunisian and one Algerian) with recessive HSP linked to chromosome 8p11-8q13 have been published. The HSP locus was mapped in a region of 32.2 cM flanked by the markers PLAT and D8S279.
In the current study, we report a small Italian RHSP family linked to chromosome 8. Using additional markers located between PLAT and D8S279, we were able to refine the HSP region.
Negative lod scores were obtained in the two-point linkage analysis by using STR markers on chromosome 15 and 16, whereas positive lod scores were obtained for D8S509, D8S1828, D8S285, D8S1102, D8S1723, D8S260, D8S1840 and D8S279 with a maximum lod score of 1.46 at D8S260 marker. The two affected siblings were homozygous for the markers D8S1102, D8S1723 and D8S260. According to the Genethon map, haplotype reconstruction revealed that the patients shared a common 8.2 cM region of homozygosity encompassing the D8S1102, D8S1723 and D8S260 markers. Based on recombination events in our Italian family, we could map the RHSP gene between D8S285 and D8S1840, thus refining the HSP region by approximately 20 cM, from 32.2 to 12cM.

P0903. Linkage of a new locus for autosomal recessive axonal form of Charcot-Marie-Tooth disease to chromosome 8q21.3.

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We report clinical and genetic linkage analysis of large Tunisian family including 13 affected patients suffering from a particular form of Charcot-Marie-Tooth (CMT) with pyramidal feature. The inheritance was autosomal recessive. The clinical phenotype was sterotyped in all patients and characterized by onset during the first decade, a progressive course and distal atrophy in four limbs associated with a mild pyramidal syndrome. Nerve biopsy performed in two patients showed severe axonal neuropathy. Genetic linkage studies allowed to exclude linkage with known loci of different forms of CMT, familial spastic paraplegia and Juvenile Amyotrophic Lateral Sclerosis. A significant lod score obtained with marker D8S286, confirming linkage to chromosome 8q21.3. The clinical syndrome observed in this family corresponds to a new genetic form of autosomal recessive CMT.

P0904. Fine mapping of disease locus and histopatholgical studies in Russian family with autosomal dominant Charcot-Marie-Tooth neuropathy type 2F

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Charcot-Marie-Tooth disease (CMT) is the most common inherited motor and sensory peripheral neuropathy. The axonal form of CMT, or CMT type 2 (CMT2), is clinically and genetically heterogeneous with several assigned autosomal dominant loci.

Last year, at the 10th International Congress of Human Genetics in Vienna, Austria, we reported of the new locus (CMT2F) for autosomal dominant Charcot-Marie-Tooth type 2 neuropathy that maps within 15 cM region on chromosome 7q11-q21 in an extended Russian family. By this moment we performed fine mapping for CMT2F locus using set of short tandem repeat (STR) markers located inside of the previously defined region and flanking markers.
Using these additional markers we got narrowing CMT2F locus. Haplotype analysis with the new additional markers demonstrates that the disease gene maps at chromosome 7q11-q21 within reduced to 8 cM region.
Presently we are also performing histopatholgical examination of peripheral nerves in affected patients from CMT2F family and these results will be ready by the ECHG 2002 conference and can be presented at the conference too.

P0905. Axonal Autosomal Recessive forms of Charcot Marie Tooth disease: genes (GDAP1 and LMNA)frequencies and spectrum of their mutations.

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Charcot Marie Tooth Disease (CMT) is a pathologically and genetically heterogeneous group of hereditary motor and sensory neuropathies characterised by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished, in which the neuropathy is either demyelinating or neuronal. Electrophysiological studies on median motor conduction velocity (MNCV) have confirmed the distinction between the demyelinating and axonal forms of the disease. Several loci and various modes of inheritance were described: autosomal dominant, X linked and autosomal recessive (ARCMT). More than 30 loci and 10 genes are implicated. Only 3 loci were reported on the axonal form of ARCMT: 1q21, 8q13 and 19q13.
36 consanguineous families with axonal ARCMT originating from the Mediterranean basin were selected and screened by linkage analysis and homozygocity mapping to these loci. Nine families were linked to the 1q21 locus (LMNA gene), 10 to the 8q13 locus (GDPAD1 gene) and only one family was linked to 19q13. The GDAP1- and LMNA-linked forms were equally frequent, representing 25% and 28% of our series, respectively. The 19q13 locus is the rarest one (3%). Therefore, ~44% remains unlinked to any known axonal ARCMT locus. Furthermore, 5 families with demyelinating ARCMT were found to be linked to the 8q13 locus. The mutation screening in the axonal and demyelinating families is ongoing and the results will be presented at the meeting.

P0906. Clinical and genetic studies of a large pedigree with rolandic epilepsy and speech dyspraxia co-inherited as a dominant trait

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The complex relationship between epilepsy and language impairment is well known but poorly understood. Rolandic epilepsies (RE) account for about one-sixth of all childhood epilepsies. Language processing deserves particular attention in RE because discharges involve the perisylvian language areas. Consequently, several studies have shown that language dysfunction may be associated with RE. One family with nocturnal oro-facio-brachial seizures and centro-temporal epileptiform discharges associated with oral and speech dyspraxia and cognitive impairment had been described (Scheffer et al., 1995).
A new family was identified (Rudolf et al., manuscript in preparation) in which the same syndrome is inherited as a dominant trait with full penetrance. Epilepsy is of the rolandic type and language dysfunction is of the same type as in the former family. Video-EEG in the youngest patient showed asynchrone biphasic spikes predominant over the centro-temporal regions increased by sleep. The five subjects who performed neuropsychological testing showed a mental delay with significantly lower verbal performances. The main verbal deficits involved speech articulation and auditory verbal memory span.
The family is composed of eleven affected individuals, of whom ten are alive. Blood samples from all ten affected patients as well as from four unaffected members were collected. Genome screen was initiated with markers spaced regularly across the genome. Statistical studies are being performed assuming a dominant mode of inheritance with full penetrance and preliminary linkage data will be presented.

P0907. The -463G/A myeloperoxidase promoter polymorphism is associated with reduced risk for late-onset sporadic Alzheimer's disease.

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Myeloperoxidase (MPO) is a myeloid-specific enzyme that catalyses the reaction of chloride and hydrogen peroxide to yield hypochlorus acid, a strong ossidant, and its reactive by-products have been linked to DNA-strand breakage. MPO is present at high levels in circulating neutrophils and monocytes but is not detectable in microglia in normal brain tissue . However, MPO presence has been demonstraded in microglia associated with Alzheimer's disease (AD) plaques, suggesting that MPO gene expression may play a role in neurodegenerative diseases involving macrophage-microglia. One portion of the gene thought to be involved in regulation of MPO expression is the proximal 5' flanking region. The polymorphic G/A nucleotide base shift, 463 bases upstream from the transcription start site, negates the binding region for the general transcription factor SP1, and results in reduced gene expression, which would imply lower susceptibility to Alzheimer's risk.
In this work, we present a case-control study to test this hypothesis. We have examined this polymorphism in a sample of 162 characterized AD cases compared with 158 cognitively normal subjects from the same geographic background. MPO genotypes were examined by PCR-RFLP. The allele frequency for MPO -463A was found to be 22.5% for cases and 34.2% for controls (OR=0.560, 95% CI=0.40-0.8, p=0.0011). These results
suggest that MPO -463 A allele reduces the risk of the Alzheimer 's disease.

P0908. Analysis of the *GRIK1* gene in patients with Juvenile absence epilepsy

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Hereditary factors play a major role in the aetiology of juvenile absence epilepsy (JAE). Sander et al. (1997) reported an allelic association of JAE with the nine-copy allele of a tetranucleotide repeat polymorphism in the third intron of the kainate-selective GluR5 receptor gene (*GRIK1*) and supportive evidence for linkage of IGE to *GRIK1* in families of JAE probands. These findings suggest that a major genetic determinant of *GRIK1* confers susceptibility to JAE. We have sequenced the coding regions and regulatory sequences of the *GRIK1* gene in 8 JAE patients who carry the nine-repeat allele of the *GRIK1* tetranucleotide repeat polymorphism to detect a putative functional *GRIK1* mutation that is in linkage disequilibrium with the nine-repeat allele. Seven of them were derived from families showing positive evidence for linkage to *GRIK1*. Our mutation analysis of coding regions and splice junctions revealed only two silent polymorphisms (A522C and C1173T) out of the five SNPs present in public databases and no mutations affecting protein structure. No significant differences were found in the allele frequencies of the detected polymorphisms between the JAE patients and controls. High levels of sequence conservation were also found in the promoter, in the 5' and both the 3' untranslated regions and in the RNA secondary structure involved in the editing reaction. These results indicate that mutations in the coding sequences, in the intron-exon boundaries and in the main regulatory and editing regions of the *GRIK1* are not commonly involved in the aetiology of JAE.

P0909. BACE1 and BACE2: exclusion of allele association with Alzheimer's disease

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Alzheimer's disease (AD) is characterized neuropathologically by neurofibrillary tangles and senile plaques in brain. A key component of plaques is Aß, a 40-42 residue polypeptide derived from Aß-precursor-protein (APP) through proteolytic cleavage catalyzed by ß and γ-secretases. ß-secretase is the rate-limiting enzyme in this process, which represents an alternate to normal a-secretase cleavage. Sequence variation in genes BACE1 (chromosome11q23.3) and BACE2 (chromosome 21q22.3), which encode two closely related proteases that appear to act as the AD ß-secretase, may represent a strong genetic risk factor for AD. In order to address this issue, we analysed the frequencies of 2 SNPs in BACE1 (V262, dbSNP rs#638405) and BACE2 (chr.21-cSNP database #hc21s00169, dbSNP rs#12149) respectively in a community-based sample of 96 individuals with late-onset AD followed in geriatric and psychiatric clinics (mean age = 79.9; SD 9.3 ; 45% men) and 170 controls randomly selected among residents of the same community who participated in an epidemiological study of dementia and underwent extensive mental status testing (mean age = 74.7 ; SD 7.4 ; 48% men). Genotype and allele distribution in both study groups were compared using Fisher's exact test and did not demonstrate any association between AD and BACE1 or BACE2. These data do not support BACE1 or BACE2 involvement in genetic risk of late onset AD in agreement with the recently published studies (Nowotny et al., 2001, Murphy et al., 2001).

P0910. Absence of association between multiple sclerosis and the –463 promoter region polymorphism of the human myeloperoxidase gene.

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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 . Myeloperoxidase (MPO) catalyses a reaction between chloride and hydrogen peroxide to generate hypochlorous acid and other reactive compounds that have been linked to DNA damage. Reactive oxygen species generated by macrophages have been implicated in inflammatory or neurodegenerative disorders. MPO is expressed in macrophages and microglia, which play a key role in the demyelination of nerve axons in Multiple Sclerosis (MS).
A G-to-A substitution polymorphism in the promoter region of the MPO gene has been suggested in vitro studies to decrease gene transcription. We tested the association of this polymorphism with Multiple Sclerosis in a population-based case-control study of 131 cases and 163 controls from the same geographic background. We did not found an association with gender, age at onset, susceptibility to or the course of MS, according to the specific genotypes of the polymorphism of the MPO gene.

P0911. Association between some candidate regions on the chromosome 6p21 and Multiple Sclerosis: a family based study in population of the Central Sardinia, Italy

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Multiple Sclerosis (MS) is an immune-mediated disease with the complex interplay between genetic and environmental factors noted in its aetiology.
Population of Sardinia is an isolated genetically homogeneous, distinct and different from other European populations that originates from a limited number of Caucasian ancestors, it has high degree of consanguinity, low migration rate, high prevalence of MS cases (157/100000 inhabitants) and high proportion of MS cases in the young ages.
6p21 region covering major histocompartibility complex (MHC) has been identified as promising in previous genome screenings.
A haplotype-based strategy of TDT mapping was adopted. The aim of this stage of the study was to evaluate the association in the 6p21 region with MS in Sardinian population.
Blood samples were obtained from 120 recruited MS trios (nuclear MS families from the MS register, Nuoro province, Central Sardinia). Mapping in some candidate regions was performed to search for the susceptibility genes. In this analysis 7 microsatellite markers (D6S2222, D6S276, STR-MICA, MICB, TNFalfa, D6S273, DQCARII) covering of about 4 cM in the MHC region (6p21) were chosen to study candidate regions identified before and according to the prior hypothesis involving TNFalfa and MICB genes.
To test for the allelic association, we performed the classical TDT test using TRANSMIT programme (D.Clayton) that allows for incomplete data.
Analysis of linkage disequilibrium and results on allelic and haplotype association between microsatellites in study and MS will be presented. Hypothesis about the role of some small regions of MHC region in the disease aetiology to be discussed.

P0912. Linkage disequilibrium analysis indicates a multiple sclerosis susceptibility effect in vicinity to the protachykinin-1 gene (*TAC1*) on chromosome 7q21-22.

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Chromosome 7q21-22, and in particular the region surrounding *D7S554*, emerged from the recent American genome screen in multiple sclerosis (MS) as the most promising region genome-wide for harboring a disease susceptibility gene. Also in the Canadian genome screen linkage was found with this chromosome region. We tested association between *D7S554* and MS in 217 Sardinian trio MS families by the transmission disequilibrium test (TDT), and in a Northern Irish case-control study comprising 542 individuals. In both populations we found evidence for significant allelic association (*Pc* = 0.04 and *Pc* = 0.0002, respectively). In a second stage, we analysed 5 microsatellite markers in a 4 megabase interval on chromosome 7q21-22 in the same set of Sardinian families. Parental transmission of a single allele of one of these markers, i.e. *D7S3126*, was significantly distorted (*Pc* = 0.008). *D7S554* and *D7S3126* are located at distances of respectively 40 and 81 kb 5' from the startcodon of the protachykinin-1 gene (*TAC1*), and occur in strong linkage disequilibrium ($P < 10^{-7}$). Our study indicates that the previous finding of linkage with *D7S554* refers possibly to the presence of an MS susceptibility effect in vicinity to *TAC1*. In addition, a second independent association was uncovered between a microsatellite polymorphism in the plasminogen activator inhibitor-1 gene, i.e. *D7S477*, and MS. Overall, the analysis presented here may contribute to the increasingly refined genomic map of MS, and underscores the requirement for a further high-resolution screening of chromosome 7q21-22.

P0913. Susceptibility and disease progression in Portuguese patients with multiple sclerosis - study of the role of APOE and SCA2 loci

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Multiple sclerosis (MS) a is a major demyelinating disease with a highly variable clinical presentation, following a progressive or relapsing-remitting course that affects around 1:500 European young adults. Genetic factors are thought to play a role in susceptibility to MS and its progression.
In order to determine the influence of the APOE and SCA2 loci on susceptibility to multiple sclerosis and their correlation with disease severity, we studied 243 Portuguese patients, who were matched by sex, age and region of origin to 192 healthy controls. Both parents of 92 patients were also studied. We did not detect any significant difference when APOE and SCA2 allele frequencies of cases and controls were compared (McNemar's test), or when we compared cases with primary progressive versus other forms of the disease (Fisher's exact test). Disequilibrium of transmission was tested for both loci in 92 trios, and we did not observe segregation distortion for any allele at both loci.
To test the influence of the APOE epsilon 4 and SCA2 22 CAG alleles in the severity of the disease, we compared the age of onset, severity and progression rate between the groups with and without those alleles. We did not observe an association of the epsilon 4 or the 22 CAG alleles with severity or rate of progression of MS in our population.
Given the importance of gene-environment interactions in MS, it is possible that different genetic factors are relevant to its pathogenesis in different populations.

P0914. No evidence of association between alpha 2 macroglobulin gene and Parkinson's disease in a case-control sample

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Parkinson's disease is one of the most frequent neurodegenerative disorders. The pathological hallmarks of PD are: a) the presence of Lewy bodies (cytoplasmatic eosinophilic hyaline inclusions) in all affected brain-stem regions, especially the dorsal motor nucleus of the vagus; and b) a massive loss of dopaminergic neurons in the pars compacta of the substantia nigra.
Lewy body contains a variety of costituents, and its antigenic determinants can be divided into four groups: structural elements of Lewy bodies during their formation. The mechanism of Lewy-body formation, the importance of Lewy body to the pathogenesis of Parkinson's disease, and its role in the neurodegenerative process remain unknown. Alpha-2 macroglobulin (A2M) is a component of Lewy bodies; two alpha2 macroglobulin polymorphisms were described: a five nucletide deletion at the 5' splice site of exon 18 and a valine (Val) to isoleucine (Ile) exchange in aminoacid position 1000 near the thiolester active site. We present a study of these polymorphisms in a case-control sample consisting of 158 PD patients to verify if a potential functional alteration of this protein would be a risk factor for PD. No significant difference regarding allelic and genotypic distribution was found between cases and controls between early and late onset PD patients. These data suggest that these polymorphisms do not represent risk factors for PD and do not modulate age at onset of PD.

P0915. Mammalian, yeast, bacterial and chemical chaperones reduce aggregate formation and death in a cell model of oculopharyngeal muscular dystrophy

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Autosomal dominant oculopharyngeal muscular dystrophy (OPMD) is characterized pathologically by intranuclear inclusions in skeletal muscles and is caused by the expansion of a 10 alanine stretch to 12-17 alanines in the intranuclear poly(A) binding protein 2 (PABP2). While PABP2 is a major component of the inclusions in OPMD, the pathogenic mechanisms causing disease are unknown. Here we show that polyalanine expansions in PABP2 cause increased numbers of inclusions and enhance death in COS-7 cells. We observed similar increases of protein aggregation and cell death with nuclear-targeted green fluorescent protein (GFP) linked to longer vs. shorter polyalanine stretches. Intranuclear aggregates in our OPMD cell model were associated with HSP40 (HDJ-1) and HSP70. Human HDJ-1, yeast hsp104, a bacterially-derived GroEL minichaperone and the chemical chaperone DMSO reduced both aggregation and cell death in our OPMD model without affecting levels of PABP2 and similar trends were seen with GFP with long polyalanine stretches. Thus, polyalanine expansion mutations in different protein contexts cause proteins to misfold/aggregate and kill cells. The situation in OPMD appears to have many parallels with polyglutamine diseases, raising the possibility that misfolded, aggregate-prone proteins may perturb similar pathways, irrespective of the nature of the mutation or protein context.

P0916. SCA7 mouse model: understanding and suppressing polyglutamine-induced toxicity

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Nine neurodegenerative disorders are caused by expansions of CAG repeats within the coding region of the disease-causing genes, encoding polyglutamine tracts in the corresponding proteins. Among these disorders, spino-cerebellar ataxia type 7 (SCA7) is the only one affecting the retina.
To investigate the mechanisms of expanded ataxin-7 neurotoxicity, we generated transgenic mice overexpressing full-length human ataxin-7 (containing 10 or 90 glutamines) in rod photoreceptors, by using the rhodopsin promoter. Expression of the mutant protein triggers a clear, easily quantifiable, retinal dysfunction associated with neurodegeneration. Intranuclear inclusions (NIs) form by accumulation of an N-terminal cleaved fragment of mutant ataxin-7, are ubiquitinated and recruit chaperones (HDJ2, HSP70) and proteasomal subunits.
Because retina is suitable for molecular analysis and for testing therapeutic strategies, we initiated two different studies using this model.
First, we addressed in vivo normal ataxin-7 fate in presence of the mutant form, by crossbreeding SCA7-Q10 and SCA7-Q90 mice. Interestingly, photoreceptors expressing both transgenes lose rapidly, progressively and completely normal ataxin-7 protein, as shown by a complete disappearance of its cytoplasmic staining, whereas aggregation still occurs. Whether mutant ataxin-7 or any polyglutamine containing proteins induce a transcriptional repression or a specific proteolytic response is now investigated.
Second, we wanted to determine whether inducing chaperone activity could allow significant protection against polyglutamine-induced retinal toxicity in our SCA7 model. Mice overexpressing either HDJ2 or HSP70 under the same rhodopsin promoter are currently characterized. Preliminary results on mice expressing mutant ataxin-7 and HDJ2 suggest a specific partial reduction of histological abnormalities.

P0917. Ataxin-7, the gene product involved in Spinocerebellar ataxia 7, interacts with Sprouty-1 and 2: the Cbl-associated protein connection.

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Spinocerebellar ataxia 7 (SCA7) is a neurodegenerative disease caused by a polyglutamine expansion in ataxin-7. The molecular basis of SCA7 disease and the normal function of ataxin-7 remain unknown. Since SCA7 patients have retinal degeneration, we used a two-hybrid approach to screen a human retina cDNA library for ataxin-7 binding proteins and have isolated Sprouty-1. Sprouty was genetically identified as an antagonist of fibroblast growth factor signaling during tracheal branching in Drosophila. To date, four mammalian Sprouty genes have been identified. Human Sprouty-1 was the most frequently cDNA isolated in this screening. By Northern blot, we observed that Sprouty-1 is expressed in brain

and peripheral tissues. As Sprouty-2 is also expressed in brain, the subcellular localizations of Sprouty-1 and 2 were examined by immunohistochemistry in mouse brain: both are expressed in the Purkinje cells affected in SCA7. The interaction between ataxin-7 and Sprouty-1 and 2 were confirmed by GST pull-down. Sprouty associates directly with c-Cbl, a known down-regulator of receptor tyrosine kinase signaling. A short sequence in the N-terminus of the Sprouty proteins was found to bind directly to the RING finger domain of c-Cbl. Interestingly, we previously identified a variant of another Cbl-associated protein, CAP, as an ataxin-7 binding protein which colocalized with mutated ataxin-7 in neuronal intranuclear inclusions of SCA7 patients. More investigations are underway to confirm and further characterize the role of Cbl and Cbl-associated proteins in the normal function of ataxin-7 and their involvment in the pathophysiology of SCA7.

P0918. Processing of Huntington's Disease protein and formation of intracellular aggregates.

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Huntington's Disease (HD), an inherited neurodegenerative disorder, is caused by an expansion of a polyglutamine stretch localized in the amino-terminal region of huntingtin, a cytoplasmic protein of 350 kDa. The polyglutamine expansion, which modifies the antigenicity of huntingtin and promotes its aggregation, confers a gain of "toxic" property by a yet unkown mechanism. Animal and cellular model systems for HD suggest that processing of mutant huntingtin is a key event in the pathogenesis of HD, as mutant huntingtin breakdown products, which aggregate in cytoplasm and nucleus, are more harmful to neurons than the full length form. Studies on the proteolysis of huntingtin could reveal potentiel targets for therapeutic interventions by uncovering proteolytic sites. In this study, we focus on the characterization of the molecular nature of the inclusions and the proteolytic activities generating huntingtin fragments, which aggregte in neurons. We report that cytoplasmic and nuclear aggregates detected in HD brain and in a neuronal cell model of HD are made up of huntingtin breakdown products differing in size. The shorter of these fragments is the major huntingtin product that form aggregates in the nucleus, and is released by cleavage in a 10 aminoacid domain. Deletion and aminoacid replacement of the proposed cleavage domain either abolishes or diminishes cleavage. We also provide evidence that these huntingtin fragments are generated by proteases, which act in concert with the proteasome to ensure the normal turn over of huntingtin.

P0919. SCA17 - a rare form of spinocerebellar ataxia identified in Poland

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Autosomal dominant ataxias are a group of neurodegenerative disorders characterized by progressive cerebellar ataxia of gait and limbs, dysarthria and other neurological signs. Several genes have been linked to different types of spinocerebellar ataxias (SCAs). Six types of SCA (SCA1, SCA2, SCA3, SCA6, SCA7 and DRPLA) are caused by an expanded CAG repeats in coding region of corresponding genes. The frequency of particular types of SCAs present an ethnical diversity.
In 2001, a new form of spinocerebellar ataxia - SCA17, with expansion of CAG repeats in coding region of TBP gene was identified in a few Japanese families. Besides typical cerebellar ataxia symptoms, patients with SCA17 presented extrapiramidal signs and intellectual deterioration.
Analysis of CAG polymorphism in TBP gene was performed in Polish control group consisted of 100 unrelated, healthy individuals with no neurological signs. The non-pathogenic repeat number in our group has ranged from 32 to 45 CAG.
Molecular analysis among 50 Polish patients with other types of SCA excluded, revealed one familial case with expansion of CAG in TBP gene. The proband was a 38 year-old woman with ataxia, bradykinesia, parkinsonism, dystonia and dementia. Her sister presented similar features and both had cognitive disturbances. Analysis of expanded alleles showed 55 CAG repeats in TBP gene in both sisters, with existing CAA interruptions.
Up to date, SCA1 and SCA2 were the only types of SCA we identified among a large group of Polish patients. These results suggest that SCA17 may be another, rare form of spinocerebellar ataxia in Poland.

P0920. Predictability of age at onset in Huntington disease in the Dutch population

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Huntington disease (HD) is an autosomal dominant, progressive neuropsychiatric disorder with chorea, dementia and changes in personality, mood and -behavior. The disease is incurable and leads to death usually within 17 years after onset (range: 2 to 45 years). The age at onset ranges from 2 to 80 years, with a mean between 46.0 and 48.9 years and the number of repeats, in the causal CAG repeat expansion, is inversely related to the age at onset and accounts for 50-77% of the variation in age at onset.
We analysed a Dutch cohort of 755 individuals retrospectively to assess the probability of onset for any given CAG repeat.
The repeat size is the major determinant of age at onset, with a correlation of -0.74, stronger (-0.84) in paternal than in maternal inheritance (-0.64), consistent with increased repeat expansion and stronger anticipation in the paternal line. The age at onset within families was more similar, than could be explained by the resemblance of the repeat size of persons in the same family.
We hypothesised that if environmental factors were principally responsible for this familiar aggregation, one would expect a greater correlation for sibs than for parents and children. Our observations suggest that genetic factors may play a greater role in the onset of HD than a shared environment. Finally we discuss several explanations for the fact that the Dutch median age at onset for all expanded repeat sizes studied is significantly later -about 10 years- than a Canadian study.

P0921. Genetic heterogeneity of Huntington's Disease : screening of Huntington's disease-like 1 and 2 loci

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting mainly from the loss of neurons in the striatum. Patients suffer from progressive and unremitting chorea, rigidity and cognitive impairment. A CAG repeat expansion in the *IT15* gene on chromosome 4 accounts for 93% of typical HD cases in our series. Recently, 2 new mutations were reported in HD-like (HDL) patients : a 192 nucleotide insertion in the PRPN gene encoding the prion protein (HDL1) and a CTG/CAG repeat expansion in the gene encoding Junctophilin-3 (HDL2). We analyzed 74 HD patients without expansions in the IT15 and DRPLA genes for these 2 mutations. The 192 nucleotide insertion in the PRNP gene was not detected in our series. The CAG/CTG repeat at the HDL2 locus was polymorphic in the controls and ranged from 8 to 28 units with a 62% heterozygosity rate. Only one Moroccan HD patient carried 50 uninterrupted CTG/CAG repeats at the HDL2 locus. This patient, a 44 year old woman with no know family history, presents with a 2 years duration of mild choreic movements of the face and extremities and sub-cortical dementia. Enlargement of the anterior horn of the lateral ventricles was observed on cerebral MRI suggesting atrophy of the head of the caudate nucleus. Cortical atrophy was marked. In conclusion, CTG/CAG repeat expansions at the HDL2 locus account for 0.2% of our series of HDL patients while the 192 nucleotide insertion in the PRNP gene was not found. Further genetic heterogeneity is then expected.

P0922. Polyalanine accumulation and toxicity in a CAG tract disorder, Machado-Joseph Disease.

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Machado-Joseph disease (MJD) is part of a group of neurodegenerative disorders caused by the expansion of a polyglutamine-coding CAG repeat. A pathological hallmark of these disorders is the presence of intranuclear inclusions (INIs) which are aggregates of insoluble proteins including the expanded polyglutamine protein. The presence of such INIs in patient material and cellular models has lead to a cellular toxicity model. Similar INIs are found in oculo-pharyngeal muscular dystrophy (OPMD), which is caused by the short expansion of an alanine-encoding GCG repeat. The similarities between OPMD and the CAG tract disorders INIs led us to hypothesize that frameshifting events leading to polyalanine accumulation could contribute to the toxicity observed in CAG tract disorders. We previously demonstrated that such events occur in patient material and in a cellular model of MJD. Here we propose a frameshifting mechanism for CAG tracts involving RNA structure formation, similar to that previously implicated in retroviral organisms. We also investigate the role of polyalanine accumulation in the cellular toxicity observed in MJD.

P0923. Episodic Ataxia 2, Spinocerebellar Ataxia 6 and CACNA1A gene mutations.

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CACNA1A gene encodes the a1A subunit of P/Q Ca2+channels and its mutations are responsible for 3 disorders: Familial Hemiplegic Migraine (FHM), preferentially associated with missense mutations, Episodic Ataxia type 2 (EA2) and Spinicerebellar ataxia type 6, thought to be due, respectively, to premature stop mutations and to small expansions of a CAG repeat.
A mutation screening, by SSCP and DHPLC, of the CACNA1A gene, still ongoing, was performed on 26 ataxia patients from different families. So far 4 patients with a mutation of CACNA1A gene were identified. Two families showed a CAGn expansion, and 2 a previously undescribed point mutation: 1 missense and 1 deletion in frame. In addition single nucleotide substitutions have been detected in introns or in exons, without aminoacid change, possibly affecting exon splicing enhancers sites. No mutations leading to premature stop were so far identified. The clinical picture was that of EA2 or of SCA6, showing once more the wide overlap between the 2 disorders. The functional analysis of some of the above EA2 point mutations favors the hypothesis of a loss of channel function. Overall the present and literature data suggest that ataxia-causing point mutations, different from those leading to truncated protein, are relatively frequent and appear to be preferentially located in the III protein domain, while missense mutations causing FHM appear to be widely spread in all protein domains.
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P0924. Ataxin 7: A Nuclear Protein Involved In Caspase cleavage-mediated Apoptotic Cell Death

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Spinocerebellar ataxia type 7 (SCA7) is a polyglutamine disorder characterized by specific degeneration of cerebellar, brainstem and retinal neurons. Although they share little sequence homology, proteins implicated in other trinucleotide (CAG)n repeat disorders have similar attributes beyond their characteristic polyglutamine tract and neurodegenerative pathology. These attributes include a nuclear aggregation phenotype associated with apoptotic cell death and roles as caspase substrates. In this study we present evidence that ataxin-7, the product of the SCA7 gene, has a predominantly nuclear distribution in HEK 293 cells that does not depend upon on isoform, polyglutamine tract length or the presence or absence of a putative nuclear localization sequence. However, the morphology of nuclear aggregation appears to depend upon polyglutamine length. Ataxin-7 is implicated in apoptotic cytotoxicity by qualitative as well as quantitative parameters . Consistent with its nuclear localization and apoptotic propensity, ataxin-7 acts as a substrate for an activated caspase. Ataxin-7's susceptibility to caspase action was eliminated by mutation of specific residues. The data support a model whereby one of ataxin-7's roles in the nucleus is as a modulator

P0925. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington's disease.

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Huntington's disease (HD) is a progressive neurodegenerative disorder with no effective treatment. The formation of neuronal inclusions with aggregated huntingtin protein is associated with the progressive neuropathology in HD. Our previous work suggested that inhibition of huntingtin protein aggregation in patients by small molecules could be a promising therapeutic strategy (Heiser et al, 2000, Proc. Natl. Acad. Sci. USA, 97, 6739-6744). Geldanamycin is a benzoquinone ansamycin that binds to the heat shock protein Hsp90. Geldanamycin disrupts a complex consisting of Hsp90 and the heat shock transcription factor HSF1 and triggers the activation of a heat shock response in mammalian cells. In this study, we show by using a filter retardation assay and immunofluorescence microscopy that treatment of mammalian cells with geldanamycin at nanomolar concentrations induces the expression of Hsp40, Hsp70 and Hsp90 and inhibits HD exon 1 protein aggregation in a dose-dependent manner. Similar results were obtained by overexpression of Hsp70 and Hsp40 in a separate cell culture model of HD. This is the first demonstration that huntingtin protein aggregation in cells can be suppressed by chemical compounds activating a specific heat shock response. These findings may provide the basis for the development of a novel pharmacotherapy for HD and related glutamine repeat disorders.

P0926. Haplotypes in five Portuguese DRPLA families

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The spinocerebellar ataxias (SCAs) are neurodegenerative disorders clinically and genetically very heterogeneous. Dentatorubropallid oluysian atrophy (DRPLA) is a type of SCA, characterized by gait ataxia, myoclonic epilepsy and dementia, due to a (CAG)n expansion on chromosome 12p13. DRPLA is prevalent in Japan, but several families of non-Japanese ancestry have been identified. Expanded DRPLA alleles of Asian and Caucasian ancestry share a common haplotype, which is associated with longer repeats commonly found in Asians. Associations between prevalence of dominantly inherited SCAs and frequency of large normal alleles have indicated that these may contribute to the generation of expanded alleles. We identified five families with DRPLA in Portugal. Interestingly, no association between the frequency of DRPLA and the frequency of large normal alleles was found in the Portuguese population. To identify the origin of the expanded DRPLA alleles in Portuguese we studied two previously reported intragenic polymorphisms in introns 1 (A1010G; system A) and 3 (T1865C; system B). Polymorphisms were detected by PCR-SSCP or/and sequencing. We found that all expanded DRPLA alleles in Portuguese share the same polymorphism in intron 3, which is common to that found in Asian expanded chromosomes. We are presently assessing the phase of expanded alleles with system A. Our preliminary results, thus, seem to indicate that expanded DRPLA alleles in Portuguese share a common haplotype.

P0927. Familial essential tremor is not associated with SCA 12 mutation in southern Italy

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Autosomal dominant cerebellar ataxias (ADCA) are a heterogeneous group of hereditary neurodegenerative disorders characterized primarily by progressive cerebellar ataxia and classified clinically in various subgroups. Recently, Holmes et al. identified in a large American pedigree of German descent, a novel form of ADCA termed spinocerebellar ataxia 12 (SCA 12). SCA 12 was mapped on chromosome 5q31-33. Affected subjects had expansions containg 66 to 78 CAG repeats in the 5'-untranslated region of the gene encoding PPP2R2B, a brain-specific regulatory subunit of protein phosphatase PP2A, whereas controls had 7 to 28 repeats. SCA 12 is a slowly progressive ADCA that differs from other SCAs becouse it typically begins with tremor of head and arms, often diagnosed as essential tremor (ET). We conducted a study to screen individuals who presented with familial ET for SCA 12 mutation. Thirty index cases (12 men and 18 women, from families) with familial ET underwent to DNA analysis for the SCA 12 mutation. As controls we enrolled 58 (23 men and 35 women) healthy subjects. All cases and controls were from the same ethnic and socio-economic background (Southern Italy). The range of allele size was 9 to 21 in both patients and controls; the most frequent allele was the 10 repeat allele (44.8% among controls, 43.3% among patients) no patient with ET presented a repeat larger then 19. In the current study, no patient with familial postural and tremor of head or arms harboured the SCA 12 mutation confirming that ET and SCA 12 are distinct diseases.

P0928. A Family with Alzheimer's Disease Caused by a Novel APP Mutation (Thr714Ala)

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Despite the fact that APP mutations were the first described cause of Alzheimer`s Disease relatively few pathogenic mutations have been described. Here we describe the occurrence of a novel APP mutation (T714A) in an Iranian family with documented affected individuals in 3 generations. The phenotype in this family is similar to that of many of the other families with APP mutations, with an onset age In the mid 50.

P0929. Mutations in the parkin gene in patients from Russia is associated with parkinsonism

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Until now only two genes have been associated with PD coding for the proteins α-synuclein and parkin. Exonic deletion of the parkin gene and parkin mutations have been assocoated with autosomal recessive juvenile parkinsonism (with onset before age 40). We studied parkin gene mutations in 55 patients from Moscow. 1, 2, and 7 exones were examined by SSCP analysis. We found an aberrant band pattern in exon 1 in 3 patients, in exon 2 in 1 patient and in exon 7 in 1 patient. Daughter of patient with alteration in exon 7 has the same pattern.
Subjects were screened for mutations in the PARK2 gene with use of a semiquantitative PCR assay that simultaneously amplified several exons too. We found alterations of gene dosage in 5 of 55 Moscow's patients with PD. Mutations included heterozygous deletion of exons 4, 10 and 12, homozygous deletion of exon 5.
Among the 55 patients with early-onset Parkinson disease, was founding 10 (18%) PARK2 mutations. At present time our workgroup continue searching mutations in other exons.

P0930. Parkin mutations are frequent in patients with isolated early-onset parkinsonism

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Parkin gene mutations have been reported to be a major cause of early onset parkinsonism in families with autosomal recessive inheritance and in isolated juvenile-onset parkinsonism (age at onset <20 years). However, the precise frequency of parkin mutations in isolated cases is not known. In order to evaluate more accurately the frequency of parkin mutations in patients with isolated early onset parkinsonism, we studied 146 patients of various geographical origin with an age at onset <45 years. All were screened for mutations in the parkin gene with the use of semi-quantitative PCR combined with the sequencing of the entire coding region. We identified parkin mutations in 20 patients including 4 new exons rearrangements and a new missense mutation. Taken together with our previous study (Lücking et al. 2000), these data show that parkin mutations account for at least 15% (38/246) of early-onset cases without family history, a proportion significantly decreasing when age at onset increases. There were no clinical group differences between parkin cases and other patients with early onset parkinsonism. However, a single case presenting with cerebellar ataxia several years before typical parkinsonism allows to extend the spectrum of parkin disease.

P0931. Mutations in the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) are associated with axonal or demyelinating recessive Charcot-Marie-Tooth disease (CMT).

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Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. The disease can be demyelinating as well as axonal and is inherited as an autosomal dominant, autosomal recessive or X-linked trait. Mutations in the ganglioside-induced differentiation-associated protein 1 gene (GDAP1) were recently shown to be responsible for recessive CMT4A (chromosome 8q21.1). GDAP1, comprising an ORF of 1077 nucleotides, encodes a protein of 358 amino acids containing a glutathione S-transferase (GST) domain. GST enzymes are known to function in antioxidant pathways and in detoxification. Therefore, GDAP1 might play a role in protecting myelin membranes against free radical-mediated damage, to which it is susceptible. We screened the GDAP1 gene for the presence of mutations in 7 recessive CMT families compatible with linkage to CMT4A. A homozygous nonsense mutation in exon 5 of GDAP1 (c.581C>G, S194X) was found in a consanguineous Moroccan family. Clinical, electrophysiological and neuropathological examination of the patients showed an axonal CMT phenotype.
A homozygous 1 bp deletion in exon 6 (c.786delG, G262fsX284) of GDAP1 was identified in two probably related Turkish families. Electrophysiological as well as neuropathological data support a demyelinating CMT phenotype. In a Turkish consanguineous family a homozygous missense mutation (c.844C>T, R282C) in exon 6 of GDAP1 was detected. Electrophysiological examination of the patient revealed an axonal CMT phenotype. In conclusion, we detected 3 distinct homozygous GDAP1 mutations (a nonsense, a frameshift and a missense mutation) in families with recessive CMT. The CMT phenotype associated with GDAP1 mutations can be axonal as well as demyelinating.

P0932. Charcot-Marie-Tooth disease type 1B (CMT1B): GFP is a versatile tool to visualize in vivo effects of Myelin Protein Zero (P0) mutations.

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P0 is a well characterized cell adhesion molecule playing a crucial role during myelination in the peripheral nervous system. GFP is a helpful tool to observe cellular events in vivo and is frequently used for this purpose in various cells or even organisms. P0 mutations cause Charcot-Marie-Tooth disease type 1B (CMT1B), Dejerine-Sottas-Syndrome (DSS) and congenital hypomyelination (CH), diseases of widely varying phenotypes, even if the same position is substituted by different amino acids. We used two insect cell lines, S2 and HighFive, in order to develop an adhesion test system to determine in vivo effects of P0 mutations. We analyzed 3 pathogenic mutations for their effect on adhesion capability in a S2 cell system and found a direct correlation of adhesion capability and severity of the disease phenotype. Subsequently we constructed fusion proteins with GFP for P0wt and the Ala221fs mutation to visualize the effect in vivo both on the intracellular localization and on the changes in the adhesion capability. The GFP-P0wt fusion revealed the expected adhesion capability and moreover the membrane insertion of the fusion protein was clearly visible using a fluorescent microscope. The Ala221fs mutation is expressed, however, no membrane insertion takes place. This may explaine the lack of its adhesion capability. Hence, this system is suitable to predict the severity of the phenotype based on expression of in vitro mutated P0 and to visualize the effect of mutations in vivo.

P0933. Genetics of X-linked congenital cerebellar atrophy(XCA)

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X-linked congenital cerebellar atrophy (XCA) is a rare genetic disorder characterized by severe hypotonia at birth, delayed motor development, dysarthria, slow eye movements and nonprogressive ataxia with normal/borderline intelligence. Global cerebellar hypoplasia is generally evident after age 2.
The XCA locus was assigned to a large genetic interval of 54cM on Xp11.21-Xq24, flanked by markers DXS991 and DXS1001 (Illarioshkin et al 1996)
In two additional XCA families an exclusion mapping strategy was adopted,
reducing the critical interval to two subregions: Xp11.21-q21.33 and Xq23-q24 flanked by markers DXS991-DXS990 and DSX424-DXS1001 respectively (Bertini et al 2000).
We have been initially focusing on the Xp11.21-Xq13 subregion. Genes or transcripts identified by bioinformatic tools, have been screened by RT-PCR to test their expression profile in the cerebellum and other tissues. Out of 77 genes, 59 have been found positive for cerebellum expression. Candidate genes have been selected and tested for mutation screening by DGGE, DHPLC or direct sequencing,according to the following criteria:
1)genes or their homologs strongly expressed during cerebellar development:EPLG2, LMO6
2)genes in which mutation or disruption resulted in cerebellar defects in either human pathology or animal models:CXCR3, NDUFA1
3) Xq deletion associated with cerebellar hypoplasia.(AR and OPHN1 genes are deleted resulting in a contiguous gene syndrome with mental retardation and androgen insensitivity) The involvement of OPHN1 gene in XCA was also excluded.
Additional XCA families are being recruited to further narrow down the critical interval.
The project is funded by Italian Telethon (492/B)

P0934. A novel PLP mutation in X-linked hereditary spastic paraplegia (SPG2) further broadens the extent of heterogeneity at this locus.

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Hereditary Spastic paraplegias (HSP) are a large group of genetically heterogeneous neurodegenerative disorders. The disease is characterized by progressive lower limb spasticity. Three X linked HSP loci have been identified and mutations in the proteolipid protein (PLP) gene have been identified in families linked to the uncomplicated SPG2 locus on Xq22. The PLP gene encodes 2 myelin proteins in the CNS, PLP and DM20 by alternate splicing. Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive disorder which is also caused by PLP mutations. PMD normally manifests in infancy or early childhood with nystagmus and cognitive impairment with progression to severe spasticity and ataxia. The most common mutation in PMD is a duplication of the entire PLP gene, resulting in a mild phenotype, while point mutations often result in a more severe disease.
Here we present findings in a large French Canadian family with a complicated X-linked recessive HSP linked to the SPG2 locus. The proband has been wheelchair-bound since the age of 3 years and at 11 years old he presents a severe spastic paraplegia, mild upper limb spasticity and mild developmental delay. Mutation analysis of the PLP gene identified a segregating mutation in the initiation codon. This G to A mutation is expected to result in complete absence of PLP. Mutations in this codon have been identified in PMD patients with mild forms of the disease, but not in HSP, thus extending the spectrum of mutations found in these allelic disorders and indicates further clinical heterogeneity than previously thought.

P0935. Refined SPG11 locus in autosomal recessive hereditary spastic paraplegia with thin corpus callosum

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Objective: To study nine Italian families with Hereditary Spastic Paraplegia (HSP) and thin corpus callosum (TCC).
Background: HSP is a genetically heterogeneous group of upper motor neuron syndromes and has been divided into pure and complicated forms. Both forms are classified by inheritance: autosomal dominant (AD), recessive (AR), X-linked. Complicated HSP is mainly recessive and five loci have been mapped. A locus on chromosome 15q13-15 (SPG11) has been identified in Italian and American pedigrees and in Japanese AR-HSP+TCC families.
Methods: We studied a total of 39 individuals from nine non-consanguineous Italian families with AR-HSP+TCC. Genetic linkage analyses were carried out with polymorphic DNA markers of 15q13-15 using an ABI 3100 Sequencer. Pairwise LOD scores were obtained using the FASTLINK version of the MLINK program. Multipoint LOD scores were generated by use of SIMWALK2.
Results: Linkage with the SPG11 region could neither be confirmed nor excluded with certainty in five families. Three families showed evidence for linkage to the SPG11 locus. Analysis of recombination events combined with data from published pedigrees narrowed the SPG11 locus to a 17.7 cM region. The upper extent of the region is between markers D15S1007 and D15S971, while the lower extent of the region is between markers D15S659 and D15S978. In one family, linkage to SPG11 could be excluded, supporting genetic heterogeneity.
Conclusions: Our analyses suggested that AR-HSP+TCC is more frequent than previously believed in Italy, narrowed the SPG11 locus, and corroborated genetic heterogeneity in AR-HSP.
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P0936. Autosomal recessive ataxias: a new gene – aprataxin – responsible for ataxia-ocular apraxia 1, and a new locus on 9q34

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The newly recognized ataxia-ocular apraxia 1 is the most frequent cause of autosomal recessive ataxia in Japan and is second only to Friedreich ataxia in Portugal. It shares several neurological features with ataxia-telangiectasia, including early onset ataxia, oculomotor apraxia and cerebellar atrophy. However, it does not share its extraneurological features (immune deficiency, chromosomal instability and hypersensitivity to X-rays). AOA1 is also characterized by axonal motor neuropathy and the later decrease of serum albumin levels and elevation of total cholesterol. We have identified the gene causing AOA1 and the major Portuguese and Japanese mutations. This gene encodes aprataxin, a new and ubiquitously expressed protein, comprising three domains that share distant homology with the amino-terminal domain of polynucleotide 5'-kinase 3'-phosphatase (PNKP), with histidine-triad (HIT) proteins and with DNA-binding C2H2 zinc-finger proteins, respectively. The results suggest that aprataxin is a nuclear protein with a role in DNA repair, reminiscent of the function of the protein defective in ataxia-telangiectasia, but would cause a phenotype restricted to neurological signs when mutant.
A second AOA locus - on 9q34 - was initially identified in a Japanese and a Pakistanese family. We have since then identified families from Portugal, France, Algeria and Turkey. In most cases, we found an association with elevated serum alfa-fetoprotein, adolescent age of onset, and ocular apraxia defined by hypometric saccades. The critical interval was reduced to a 3 cM region.

P0937. Infantile Onset Progressive Ascending Spastic Paralysis is Linkes to ALS2/Alsin Gene on 2q33-35

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We report on 15 patients from 10 families (4 familial and 6 sporadic forms) who presented severe progressive ascending spastic paralysis. They were considered normal at birth and spastic paraplegia initiated during the two first years of life. Weakness and spasticity extended to upper limbs around the age of 7-8 years. The patients were all wheel-chair bound by the age of 10 years and during the second decade the disease progressed to tetraplegia, anarthria, dysphagia and slow eye movements. Muscle Biopsy and EMG were normal, PEM showed abolition of corticospinal response en contrast with normal SEP. Genotyping analysis excluded linkage to previously reported loci for dominant as well as and recessive Hereditary Spastic Paraplegia and established linkage to ALS2 locus on chromosome 2q33-35 with a lod score of 6.66, raising the possibility that this infantile onset ascending spinobulbar paralysis is allelic to the condition previously reported as juvenile amiotrophic lateral sclerosis (ALS2). Therefore, we tested as gene candidate the ALS/Alsin gene recently reported mutated in 3 families consanguineous Arabic families with ALS2 and 1 consanguineous Saudi family with a childhood-onset primary lateral sclerosis (PLS). Alsin mutations were found in 4 families: 3 deletions and 1 splice site mutation leading to truncated Alsin protein. In the six remaining families absence ALS2/Alsin gene mutation suggest an heterogeneity in infantile onset progressive ascending spastic paralysis.

P0938. *De Novo* mutations in the sodium channel gene SCN1A cause severe myoclonic epilepsy of infancy

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Severe myoclonic epilepsy of infancy (SMEI) is a rare disorder occurring in patients without a family history of a similar disorder. Early manifestations of the disease are tonic, clonic and tonic-clonic seizures occurring within the first year of life. These seizures are often prolonged, generalized and associated with fever. Later in life, SMEI patients suffer from afebrile seizures, including myoclonic, tonic-clonic, absences, simple and complex partial seizures. Early psychomotor and speech development is normal, but in the second year of life developmental stagnation occurs. Patients often become ataxic and speech development is delayed. In general, SMEI is very resistant to all anti-epileptic drugs. A mild type of epilepsy associated with febrile and occasionally afebrile seizures in adulthood is generalized epilepsy with febrile seizures plus (GEFS+). Missense mutations in the gene coding for a neuronal voltage-gated sodium channel α-subunit (SCN1A) were identified in families with GEFS+. Since both GEFS+ and SMEI show fever-associated seizures we screened 13 unrelated SMEI patients for mutations in SCN1A. Mutation analysis was performed using DHPLC and DNA sequencing. We identified a single heterozygous mutation in each patient: 4 frameshift, 2 nonsense, 2 splice donor and 5 missense mutations. Using a multi-allelic marker we genotyped the patients and the parents to confirm paternity. Sequencing demonstrated that all mutations are *de novo* mutations. Pyrosequencing was used to confirm that none of the mutations occurred in 184 control chromosomes.

P0939. Disruption of the serine threonine kinase 9 gene (STK9) as the cause of X-linked infantile spasms

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X-linked infantile spasms (ISSX) or West-syndrome (WS) are characterised by onset of generalized seizures between 3 and 7 months of age, hypsarrhythmia on the electroencephalogram (EEG) and mental retardation (MR). In the pursuit of the ISSX/WS gene we have acquired two patients with balanced translocations 46,X,t(X;6)(p22.3;q14) and 46,X,t(X;7)(p22.3;p15) and clinical features of severe ISSX/WS. Both patients had early onset of seizures at age 2-3 month, and developmental arrest with profound mental retardation. Cloning of the X-chromosome breakpoints revealed the serine threonine kinase 9 gene (STK9, Montini et al. Genomics 51:247-433, 1998) to be interrupted by these rearrangements. Mutation screening of the 21 exons of the STK9 gene in three ISSX families was negative. In the meantime the ISSX/WS gene interval was refined on one Canadian family to ~7cM region in Xp21.3-Xp22.1 (Bruyere et al. Clin Genet 55:173-181, 1999); importantly, excluding the STK9 gene. More recently, the ISSX gene (MIM # 308350) has been identified from this interval (J. Gecz, unpublished). Based on the identical phenotype of our two patients with balanced X-autosome translocations we suggest, that there are at least two genes for ISSX on the human X-chromosome. We propose, that lack of a functional Stk9 protein is responsible for a severe form of West-syndrome.

P0940. Implication of the GABAA receptor in familial epilepsy with febrile seizures

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GABAergic neurotransmission is known to be involved in epilepsy since many decades. We report a K289M mutation in the GABAA

receptor gamma2 subunit gene (GABRG2) that segregated in a family with a phenotype closely related to Generalized Epilepsy with Febrile Seizures Plus (GEFS+), an autosomal dominant disorder associating febrile seizures and generalized epilepsy that had so far been linked only to mutations in sodium channel genes. The K289M mutation affects a highly conserved residue located in the extracellular loop between transmembrane segments M2 and M3. Functional analysis in Xenopus oocytes confirmed the genetic evidence of the implication of this mutation. The K289M mutation caused a dramatic decrease in the amplitude of GABA-activated currents compared to wild-type receptor. This study provides the first genetic evidence that a GABAA receptor is directly involved in a human idiopathic epilepsy.

P0941. Molecular characterisation of a 11q14.3 microdeletion associated with leukodystrophy of unknown cause.

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We have previously described a de novo 11q14.3 microdeletion in a boy with leukodystrophy of unknown cause and oculocutaneous albinism. The detection of this chromosomal rearrangement revealed a new chromosomal region susceptible to bear a gene involved in the pathogenesis of leukodystrophy. We have molecularly characterized this microdeletion in order to identify the causative gene. Eighteen polymorphic microsatellite markers mapped in 11q14.3 were selected to test the patient for hemizygosity. The maternal alleles of two of them, D11S1780 and D11S1367, were deleted, while eleven were not and five were uninformative. The genetic size of the defined region is 2.4 cM. We have constructed a contig of BACs encompassing the entire region of interest that allowed us to obtain a physical map of this region. Only one gap of unknown size persists among this BAC contig. The DNA of several BACs from this contig was extracted and used as molecular probes for FISH analysis on patient's chromosomes. The size of the deleted region was reduced to 2 Mb. Sequence annotation of this region was performed in order to identify candidate genes implicated in leukodystrophy determinism. We are now testing the potential implication of these candidate genes both in our patient and in a group of children with leukodystrophy of unknown cause. Identification of the causative gene will be useful for elucidation of the molecular bases of leukodystrophies, the cause of which remains unknown in 30% of cases.

P 20. Prenatal and Perinatal Genetics

P0942. Prenatal Diagnosis of Meckel Gruber Syndrome and Dandy-Walker Malformation in four affected consecutive siblings, the fourth one diagnosed at 22 weeks' of gestation

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Meckel Gruber syndrome (MGS) is a rare autosomal recessive disorder characterized by posterior encephalocele, post-axial polydactyly and dysplastic, polycystic kidneys.
We report a 23 weeks' old male fetus affected by Meckel Gruber syndrome. Posterior encephalocele, post-axial polydactyly, Dandy-Walker malformation were observed in the ultrasonographic (USG) examination at 22 weeks' of gestation but lisencephaly and holoprosencephaly were demonstrated with post mortem magnetic resonance (MR) before the autopsy. After the termination of the pregnancy, polycystic dysplastic kidneys were also noted in the post mortem investigation.
The proband was the fourth pregnancy of a consanguineous family which the all three siblings were also affected similarly. Interestingly, the 2 years old affected sister and 23 week's old male fetus had the same Dandy-Walker malformation.
It was concluded that, family history, sonographic examination, measurement of serum or amniotic fluid alpha-fetoprotein have crucial importance in genetic counselling and prenatal diagnosis of MGS.

P0943. Anxiety of mothers participating in second trimester screening for Downs syndrome

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Objective : To test the anxiety during second trimester serum screening for Downs syndrome, we compared 253 mothers with false positive result of second trimester screening and 183 mothers , who underwent invasive prenatal diagnosis because of age .
Methods: We interviewed all mothers 3-8 years after birth of their healthy baby and asked them about their anxiety during the time of waiting on the result of karyotype obtained from amniotic cells. Women were asked to choose one number of 7 point scale (0-6), the individual numbers marked the level of anxiety (stress).We asked them also about their attitudes towards eventual termination of pregnancy (TOP) in the speculative case, that karyotype would be abnormal and we asked them about their opinion of genetic investigation during pregnancy.
Results: Median of anxiety features in the group of mothers with false positive results was 4, median in second group was 3. The result is statistically significant (p value <0,0005). Statistically significant is also the difference in the number of women with positive attitudes towards eventual TOP (63,2% vs. 75,4%, p value = 0,020) and suitability of genetic investigation during pregnancy.(83% vs. 91,8%, p value =0,018).

P0944. The fetal neurology clinic. A multidisciplinary approach for the treatment of the fetus with suspected nervous system pathology.

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Congenital nervous system anomalies are the second most common congenital anomalies and the most frequent cause of malpractice litigation in the field of prenatal diagnosis. The difficulty in diagnosis resides in various factors: difficult approach to the fetal brain by standard ultrasound, late development of some brain structures and possible lack of neuroanatomic and neuropathologic experience by most sonographers. We reviewed all the cases referred to the Fetal Neurology Clinic at The Edith Wolfson Medical Center during a 2-year period between November 1999 and October 2001.
During this period 128 patients were referred to the Clinic, and 187 examinations were performed (1.46 examinations/patient).The ultrasound findings and the patients' follow up are summarized. Our team recommended continuing the pregnancy with regular follow up in 59.4% of the patients, in 15.6% the findings were so serious that termination of pregnancy was advised and in 10.6% we advised the patients to consider termination of pregnancy, 7.8% of the patients were referred for MRI and 7% for amniocentesis.
The fertile interaction between the different sub-specialties involved in counseling helped in the differentiation between normal and pathologic cases. In cases with suspected pathology, the team discusses with the parents the possible implications and the meaning of the diagnosis, based on the conjoined clinical experience of the team. This approach enables optimal perinatal management of the pregnancy.

P0945. Detection of fetal cell in a minimal amount of maternal blood using HbF staining and polymerase chain reaction

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The available fetal cells in maternal blood give a potential source for the detection of fetal abnormalities without risk to the fetus. We attempted to detect fetal cells and fetal DNA in a minimal amount

of maternal blood obtained from forearm and fingertip. Fetal cells were isolated from 1 µl of maternal blood by fetal hemoglobin (HbF) staining and microdissection, while DNA was extracted from 200 µl of maternal blood. The isolated fetal cells and DNA were analyzed by polymerase chain reaction using Y chromosome specific primers. These techniques correctly identified the sex of the fetus in all pregnancies tested. Our study showed that fetal DNA could be detected in maternal blood obtained from fingertip at 6 weeks of gestation. The findings suggest that prenatal diagnosis can be carried out as early as 6 weeks of gestation with a small amount of maternal blood using the inexpensive techniques mentioned above.

P0946. Trends In Live Birth Prevalence Of Congenital Anomalies. 1979-1999 : The Impact Of Prenatal Diagnosis

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Objectives: To describe the impact of prenatal diagnosis on the birth prevalence of congenital anomalies during 21 years (1979-1999) in a well defined population.
Design: A descriptive population-based study.
Setting: Northeastern France (13,500 births per year).
Methods: Analysis of data from multiple sources on births and terminations of pregnancy after prenatal diagnosis of congenital anomalies in 265,679 consecutive pregnancies of known outcome. The study period was divided into 3 subgroups 1979-88, 1989-93 and 1994-99.
Results: Between 1979 and 1988 and 1993 and 1999 prenatal detection of congenital anomalies increased from 11.7% to 25.5% and to 31.9%. Termination of pregnancy (TOP) increased in the same proportions during the 3 time periods. However the increase of TOP was much higher for chromosomal anomalies than for non chromosomal congenital anomalies : 21.7, 43.9 and 64.0 vs 4.8, 7.3, and 10.2 respectively. The birth prevalence of Down syndrome fell by 80% from 1979-88 to 1994-99. Sensitivity of prenatal detection of congenital anomalies and TOPs were lower for isolated cases (only one malformation present in the fetus) than for multiple malformations in the same fetus. Sensitivity varied with the type of malformations : it was high for neural tube defect (79.7%) and urinary anomalies (54.8%) and low for congenital heart defects (25.3%) and for oral clefts (27.6%)
Conclusions: The introduction of routine prenatal diagnosis has resulted in a significant fall in the birth prevalence of congenital anomalies. However this fall varied with the types of congenital anomalies.

P0947. Congenital deficiency of alpha fetoprotein and associated chromosomal abnormality in the placenta

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The role of alpha-fetoprotein (AFP) is unknown for the most part. Several functions of AFP during fetal life have been suggested: regulation of osmotic pressure, mediation of the immune system and growth control. In this report we describe two cases of congenital absence of AFP that were identified by the current methods of detection. The pathological examination results, including an immunohistochemical stain, which refine the levels of AFP detected by the biochemical studies, are enclosed. In order to exclude chromosomal anomalies in the placentae, we preformed complete genomic hybridization analysis on both placentae.Both placentae showed monosomy 16, which was confirmed by FISH. It has been reported that tissue-specific expression of the AFP gene is strongly stimulated by an enhancer present 3.3 to 4.9 kb upstream of the transcription initiation site mapped to 16q22.3-q23.1.An overview of the molecular biology of AFP production is set forth. An explanation is suggested for the lack of symptoms in a newborn of undetected levels of AFP and the mechanism by which this condition might occur. This may shed light on the mechanism by which this rare condition is generated and the lack of any symptoms in affected newborns

P0948. The incidence of confined placental mosaicism in non-cultured cells of human spontaneous abortions

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One of the most important conceptions over the past few years in interpretation of the pathogenesis of abnormal intrauterine human development is the statement about possible discrepancies between karyotype of cells of embryonic and placental origin. During prenatal diagnosis the incidence of confined placental mosaicism is evaluated usually about 1-2%. In order to determine the influence of distribution of cells with chromosomal abnormalities in placental tissues on arrest of embryo development the non-cultured cells from cytotrophoblast and extraembryonic mesoderm of 28 first trimester internal abortions were studied by interphase FISH analysis with centromere-specific DNA probes for each chromosome. 7 discrepancies (25%) between karyotypes of two studied tissues were found. Cell lines with trisomy 7, trisomy 8, monosomy 15 were confined to cytotrophoblast whereas the karyotype of extraembryonic mesoderm cells was normal. One embryo has mosaic monosomy 7 in cytotrophoblast only but in mesoderm the tetraploidy/diploidy mosaicism was detected. Two abortions have chromosome aberrations both in cytotrophoblast and extraembryonic mesoderm but in former the chromosomal abnormalities were in mosaic form with normal cell line whereas in later only the cells with abnormal karyotype were detected. One embryo has 45,X/46,XY/46,XX/47,XXY karyotype in cytotrophoblast and 45,X/46,XY/46,XX in mesoderm. Our data based on analysis of non-cultured cells indicates that the tissue-specific compartmentalization of cell lines with chromosomal abnormalities may have a significant influence on pathogenesis of early embryo development.

P0949. Fetal erythroblasts are not the source of cell free fetal DNA in the maternal circulation

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Fetal cells, specifically fetal erythroblasts, as well as cell free fetal DNA are present in the maternal circulation. Both are currently being investigated as a means for the non-invasive risk free analysis of fetal genetic traits. The origin of cell free fetal DNA is currently unclear. It has been proposed that trafficking fetal erythroblasts may be one source. This is partly due to the apoptotic character that a significant proportion of fetal erythroblasts display, and since elevations in both fetal erythroblast numbers as well as cell free fetal DNA concentrations have been noted in several pregnancy related pathologies. Our examination of fetal cell trafficking and release of cell free fetal DNA in normal and affected pregnancies indicates that no correlation exists between these two fetal molecular and cellular species. This is most evident in pregnancies affected by onset of pre-term labour, where significant elevations in cell free fetal DNA concentrations were detected without any concomitant elevation in fetal erythroblast numbers. Our data therefore suggest that an alternative cell type is the source of cell free fetal DNA. Furthermore, it appears that the release of cell free fetal DNA from this cell type is affected by pathological placental conditions which are not associated with an increase in fetal cell trafficking.

P0950. Numerous NRBCs in the fetal circulation display an apoptotic phenotype

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Non-invasive risk free prenatal diagnosis can be achieved by the analysis of intact fetal cells and cell free fetal DNA in the maternal circulation. The relationship between these two fetal cellular and molecular analytes is unclear. Recent data have shown that apoptotic fetal cells can be detected in plasma of pregnant women and that many fetal nucleated red blood cells (NRBCs) in the maternal circulation were TUNEL positive. It has, therefore, been suggested that the maternal immune system may clear NRBCs by apoptosis and that this leads to the production of circulatory fetal DNA. On the other hand, this apoptotic phenotype may be associated with erythroid differentiation and enucleation.
For this purpose we have examined whether apoptosis occurs in fetal

NRBCs that have crossed into the maternal circulation or rather if this is a physiological phenomenon of NRBC maturation which occurs when these cells are still in the fetal circulation.
Our study, performed on fetal blood samples (n=12), showed that more than 60% of the NRBCs were TUNEL positive. This was true for both cord blood (n=10) samples collected at term as well as those obtained from early stages of gestation (13-16 weeks: n=2). Virtually none of the fetal lymphocytes exhibited such a characteristic.
The apoptotic phenotype displayed by NRBCs therefore does not appear to be due to an interaction with the maternal immune system. This phenotype may, however, help account for the poor analysis of NRBCs by FISH or PCR.

P0951. Cerebral malformations-positive diagnosis

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Purpose: the diagnostic froming of ultrasound detected lesions, establisment of a correlation between ultrasound and clinical sings, establisment of evolutive stages and terapeutical indications for a selective group of premature new-born.
Material and method: The study was made at Neonatology and Puericulture Clinic for a eight years period (1994-2001). In the studied group was involved 27 patients with central nervous system malformation which was included in this group by anamnestic, clinical, ultrasound, tomographic criterions . Ultrasound was essential method for establish positive diagnosis. The examination was performed with “Sonoage 1500” after a standard protocol of examination wich included coronar and sagital sections.
Results: detected lesions was: cranio-vertebral disrafies 15 cases (55,55%), sindrom Arnold Chiari II - 3 cases (11,11%), sindrom Dandy-Walker - 3 cases (11,11%), corpus calosum agenesis - 5 cases (18,51%), Galen vein malformation - 2 cases (7,40%), arahnoidian cystes - 2 cases (7,40%).
Most lesions was associated: myelomeningocele - corpus calosum agenesis, myelomeningocele - malformation Arnold Chiari II, sd. Dandy- Walker - corpus calosum agenesis.
All cases of central nervous system malformation presented ventriculomegaly in moderate to severe stages - ventriculoperitoneal shunt was performed in 2 cases (7,40%).
Conclusions: The most frequent malformative type was cranio-vertebral disrafies associated with Arnold Chiari malformation II and III, corpus calosum agenesis, sd. Dandy-Walker. By ultrasound, ventriculomegaly was present in all patients of this study.

P0952. Determination of RhD zygosity by real-time PCR

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Haemolytic disease of the New-born (HDN), whereby the Rhd mother develops antibodies against her RhD fetus, is still a serious obstetrical problem. In pregnancies at risk for HDN it is useful to know the RhD zygosity of the partner, since there is only a 50% chance that the fetus will at risk for HDN, if he is heterozygous. The determination of this has previously been very difficult and unreliable by conventional antibody assays.
Recent reports have indicated that RhD zygosity can be determined by quantitative fluorescent PCR or Taqman real-time PCR.
We have recently developed a multiplex real-time Taqman assay for the simultaneous analysis of the male SRY locus and RhD gene. Our studies have shown that this assay is very reliable for the analysis of both fetal sex and RhD status using cell free fetal DNA in maternal plasma.
Since one only needs to determine RhD zygosity of the male partner in pregnancies at risk for HDN, we have examined whether our Taqman assay can be used for this purpose.
In our study we examined 39 male blood samples. RhD zygosity was determined both by the ratio of RhD:SRY or RhD:GAPDH loci by Taqman PCR. Our study showed that RhD zygosity could quite clearly be classified as either RhD/RhD and RhD/Rhd by both assays. No discordant results were obtained between the two assays. Our data, therefore, suggest that real-time PCR can be used to reliably determine the RhD zygosity of male partners in pregnancies at risk for HDN.

P0953. Hirschprung’s disease or Meconium Plug Syndrome-a ten years experience

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The study’s specific objectives were: to estabilish the rate of occurancy of the meconium plug syndrome in newborne as a cause of intestinal obstruction; to certifie the role of different clinical presentations of the syndrome in a proper diagnosis; to verifie the main methods used in early differentiation from Hirschprung’s disease in neonatal periode.
The authors are studing all newborns hospitalised in our departaments presentind signs and symptoms of intestinal obstruction between 1992-2001.
Sumary of results: In the last decade the authors hospitalised 58 cases of newborns with intestinal obstructions inclouding intestinal atresia and stenosis (19 cases), meconium ileus (6 cases), necrotizing enterocolitis (3 cases), Hirschprung’s disease (10 cases), malrotation (4 cases, meconium peritonitis (3 cases) and meconium plug syndrom (13 cases). After exclouding the other causes of intestinal obstruction, the authors corelated meconium plug syndrom with prematurity (84,61%), sweat test significant for cystic fibrosis (15,38%) and hypoglicemia and encreased glucagon prouction (7,69%).
Conclusion:
1. The rate of occurency for meconium plug syndrome in our study was 22,41% inclouding all.
2. Prematurity was registred in 84,61% from all cases with meconium plug syndrome.
3. In two cases (15,38%) the sweat test was significant for cystic fibtosis.
5. Hypermagnesemia was not proved as a cause of hypomotility in any patient in study.
6. The Gastrografin enema exam was also a therapeutic method, all patients begin passing meconium spontaneously after test.
7. Rectal biopsy was capital to exclude Hirschprung’s disease.

P0954. Fetal Chromosomal Analysis of Pregnancies Following intracytoplasmic Sperm Injection with Amniotic Tissue Culture

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From January 1996 to December 2000, 98 consecutive patients who had become pregnant after Intracytoplasmic Sperm Injection **(**ICSI) were studied. Hundred and forty-two fetuses of these patients were screened with fetal amniotic tissue culture. Chromosomal anomalies were detected from 6 out of 142 (4.2%) fetuses. Anomalies were as follows, ‘46,XX / 69,XXX / 92,XXXX‘, ‘46,XY / 69, XXY / 92,XXYY‘, ‘47,XY+21‘, ‘47,XY+7‘, ‘47,XXY‘ and ‘45,X0‘. All except one pregnancies were terminated with the consent of the couples. Fetal skin fibroblast cultures were also studied after termination of the pregnancy in order to confirm prenatal diagnosis.
The prevalence of chromosomal anomalies seems to be slightly increased after ICSI, carrying the risk for transmission of chromosomal aberrations of paternal origin and a higher risk of de novo, mainly sex-chromosomal aberrations.

P0955. Isolated fetal choroid plexus cysts and association with chromosome anomalies

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OBJECTIVE: Fetal choroid plexus cysts (CPC) are commonly found at the time of a routine second-trimester scan, but there is much debate as to their clinical significance. The aims of this study were to define the incidence of CPC in an unselected population and describe their association with aneuploidy.
METHODS: 10594 pregnant women that were subjected to second

level ultrasonographic analysis at the Department of Perinatology, Zeynep Kamil Maternity and Children Hospital has been studied. 109 cases of CPCs were identified among these group (1.02%) between 16 and 22 weeks' gestation. These patients had genetic counseling, and biochemical testing and amniocentesis were offered to all patients.
RESULTS: The majority of the cases 102/109 presented only isolated CPC and the remaining 7/109 (6.5%) were associated with additional ultrasonographic findings. No relationship was found between the diameter, bilaterality or the complexity of the cyst. Aneuploidies (mostly trisomy 18) were found in 3/102 with isolated CPC (2.94%) and in 3/7 with additional ultrasonographic findings (43%). Advanced maternal age as an additional risk factor was found in only one of isolated CPC, but the remaining two had no other risks so far.
CONCLUSION: The management of isolated choroid plexus cysts remains controversial. Fetus with CPC should be examined carefully by detailed ultrasound assessment to seek for further minor malformations in a specialized center. The review of the literature show that the majority of the authors advocate amniocentesis when the CPC is associated with another ultrasound abnormality.

P0956. Short Tandem Repeats (STR)Polymorphism Is Useful in Detection of Down syndrome

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The purpose of this study was to determine the number of chromosome 21 present in the fetal cells from pregnancies complicated with Down syndrome by differentiating the chromosome 21s of different parental origins with human chromosome 21-specific DNA marker polymorphism. Forty amniotic fluid samples from pregnancies complicated with fetal Down syndrome were analyzed for D21S11 and interferon-α receptor gene intervening (IFNAR) sequence. Fluorescence-labeled polymerase chain reaction (f-PCR) was performed to amplify these sequences followed by polyacrylamide gel electrophoresis. Data were delineated with automatic DNA sequencer and 35 of 40 (87.5%) fetal Down syndrome samples analyzed for IFNAR showed 3 distinct peaks, each peak represented an individual chromosome 21, while 24 of 30 (80%) cases analyzed for D21S11 showed 3 distinct peaks. There were two Down syndrome samples showed two uneven peaks. By analyzing 98 euploid pregnancies as controls, the ratio of area under the peaks was determined to be 1.31±0.22 and 1.96±0.18 (mean ± SD) for the euploid pregnancies and pregnancies complicated by fetal Down syndrome with two peaks, respectively. Altogether 39 of 40 (97.5%) Down syndrome cases were correctly identified based on either the 3 peaks pattern in at least one of the DNA markers or the relative peak area ratio calculation. It was concluded that polymorphic DNA markers are useful in determining the number of chromosome 21 present in fetal cells. The high sensitivity suggested good prospect of this method in application for prenatal detection of chromosome 21 aneuploidy.

P0957. Attitudes of pregnant women towards the risk free prenatal diagnosis of fetal aneuploidies using fetal cells from maternal blood

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A major focus of our group is the development of a risk free non-invasive method for prenatal diagnosis of fetal aneuploidies using fetal cells enriched from the blood of pregnant women. A concern with the introduction of such a new test is that pregnant women may feel coerced to undergo involuntary testing. In order to address this issue we have examined the attitude of 145 pregnant women to the introduction of such a test, consisting of 3 groups:
1. Women with a normal pregnancy (n=97).
2. Women judged to have a high risk for a fetal abnormality and who had undergone an invasive prenatal diagnostic procedure (n=24).
3. Women who had become pregnant following IVF treatment (n=24).
In addition we examined the attitude of 68 non-pregnant women.
Our study showed that:
1. 79,3% would not feel coerced by the introduction of a risk free test.
2. 80% would be interested in first using a non-invasive test as preliminary a screening procedure.
3. If the test result was found to be normal, 84.4% of pregnant women would not elect to have further tests.
4. If the result was abnormal, 56% would elect to have the result confirmed by invasive tests.
5. Women undergoing IVF treatment, would prefer to have the result confirmed by a further non-invasive test (58.3%).
Our data, therefore, indicate that pregnant women would not feel coerced to undergo involuntary testing, and that such a risk free test would be especially welcomed by couples undergoing IVF treatment.

P0958. Interphasic Fish On 162 Uncultured Amniotic Fluids

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FISH of uncultured amniotic fluid cells and conventional cytogenetic analysis were performed on 83 pregnancies with US abnormalities and 79 on pregnancies with normal US parameters. The AneuVysion^R Assay (Vysis) with specific probes for chromosome 13, 18, 21, X and Y, was used. Amniotic fluid samples were obtained between 12 and 34 weeks' gestation, 116/162 (72%) being between 15 and 20. In cases with a sole abnormal US finding (n=24) nine aneuploidies were detected (1 tris 13, 8 tris 21). In the group with two or more malformations (n=59) there were 18 aneuploidies (9 tris 18, 3 tris 21, 2 monosomy X,1 tris 13, 2 triploidy, 1 karyotype: 48,XXY,+21). In this group conventional cytogenetic revealed also three chromosomal anomalies not detectable by FISH: a trisomy 16 mosaicism, a 4p- and a r(13). No sex aneuploidies alone were observed. In the group with normal US, 52 cases were analysed because of maternal anxiety and 27 had positive maternal serum screen or advanced maternal age. In this latter group of pregnancies only two trisomies 21 was detected. The complete concordance between FISH data and conventional karyotype analysis observed in our sample, prompt us to consider the interphase FISH as an useful tool in pregnancies with high risk of chromosomal aneuploidies. When ploidy status is known to be normal, the overall risk of chromosomal abnormalities is significantly reduced. Otherwise, the findings of three chromosomal anomalies undetectable by AneuVysion^R Assay confirms that FISH results should be complemented by the conventional chromosomes analysis.

P0959. Further experiences with the application of STR analysis for the detection of the most common trisomies by F-PCR

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OBJECTIVE: Prenatal cytogenetic analysis for trisomies requires lengthy and work-intensive laboratory procedures. A rapid method for the detection of the most common trisomies is the fluorescent PCR amplification of small tandem repeats (STR) located on the chromosomes 13, 18 and 21. This method is a potential alternative to conventional cytogenetic analysis for the screening of these disorders. Recently the method has been reported to be used as a single screening method for age-related aneuploidies. OBJECTIVE: We compared the results of the routine cytogenetic analysis of amniotic fluid and chorionic villus samples to the results of the fluorescent PCR analysis of the same samples (n=1200). METHODS: The multiplex fluorescent PCR was performed with 3 primer pairs specific for chromosome 21 (D21S11, D21S1411, D21S1312) and 2-2 primer pairs specific for chromosome 18 (D18S851, D18S51) and 13 (D13S631, D13S258). The capillary electrophoresis and fragment analysis was performed on an ABI 310 Genetic Analyser. RESULTS: We found 16 cases of trisomy 21, 10 cases of trisomy 18, 7 cases of trisomy 13 and 3 cases of triploidy. We found the same pathologic samples as the cytogenetic analysis. CONCLUSION: The multiplex fluorescent PCR analysis of STRs proved to be fast and reliable in the diagnosis of the most common trisomies. It is not replacing the

cytogenenetic analysis of prenatal samples, but in certain cases like advanced maternal age it is worth considering it as a routine alternative to it.

P0960. 12 Years experience in Prenatal Diagnosis: Report of 2000 cases

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Over 12 years, more than 2000 prenatal tests have been performed with the following indications: 1300 for chromosomal aberrations, 510 hemoglobinopathies, 65 metabolic disorders, 25 muscular dystrophy, 15 fragile X, 6 skin lesions and 1 for ataxia telangiectasia.
The indication, results and outcome of chromosomal study of 1250 amniotic cell cultures mostly at 13-15 weeks are being reported. 662 parous were tested for advanced maternal age; 15 (2.3%) were abnormal, which shows 25 fold increase over general population. The risk is even higher, 2 of 49 (4.4%) parous with advanced maternal age who had previous history of offspring with chromosomal aberration. The highest risk is among parous (15%) where one of the parents bears a balanced translocation; 3/269 (1%) of parous with history of offspring with chromosomal aberrations showed unbalanced karyotypes. There are 2 abnormal karyotypes among 35 parous with low AFP serum, and 1 among 117 pregnancies referred for various reasons including check up. Of the 30 abnormal karyotypes, 11 have tri 21, 6 tri 18, 3 mosaicisms, 2 XXY, and 8 different karyotypes. Besides chromosomal aberrations, one anencephaly, and one full mutation of FMR1 have been detected coincidentally, totalling 32 candidates for therapeutic termination. Rate of spontaneous abortion of unknown reason, two weeks following the procedure is less that 0.5 %. The final results were reported within 10-14 days. Our experience indicates that early amniocentesis is a safe, acceptable and reliable procedure for detection of fetal chromosomal abnormalitites; we recommend it strongly for at risk pregnancies.

P0961. Evalution of balanced and unbalanced chromosomal abnormalities (except classic aneuploidies) in 700 amniocenteses, after birth or termination.

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We wish present our two years experience for prenatal diagnosis since 1999. During this period, we analysed 700 amniocenteses. Amnion fluid of patients were sent to our laboratory due to three main reasons for referral; advenced maternal age, abnormal maternal serum screening test and abnormal ultrasound assesment. None of cases with chromosomal abnormalities do not include chromosomal aneuploidies for chromosomes 13, 18, 21, X, Y and pericentric inversions of chromosome 9 in these evaluation. Three reciprocal, two Robertsonian, one insertional and one de nova translocations as balanced abnormality and 4 unbalanced chromosomal abnormalities were detected in these cases. These unbalanced chromosomal anomalies were as 46,XX,der(1) add(q32-44); 46,XY, 21p+; 46,XX, +mar; 46,XX,(1qh+). Marker chromosome was orginated from chromosome 15 centromeric region which was detected by using flourescence in situ hybridization (FISH). All fetuses were examined after birth or termination. Our finding sugests that 50 % fetuses with unbalanced chromosomal abnormalities (in 2 out of 4) showed some physcial anomalies, such as low air, hydrocephalus, facial and cardiac anomalies, while none of fetuses with balanced translocations had any phenotypical abnormality.

P0962. Evaluation Of Histology And Molecular Cytogenetics Of Placenta In 56 I.u.g.r. Cases

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The role of confined placental chromosome mosaicism (CPM) in intra-uterine growth retardation (IUGR) has not yet been well established and also the involvement of uniparental disomy (UPD). The aim of the research is to understand if the IUGR, as the only foetal anomaly, is due to CPM and/or UPD. Up to now a total of 56 consecutive foetuses with IUGR (below the 10th centile) and no major morphological abnormalities were recruited for the study. All foetuses were karyotyped at birth and only two showed an abnormal chromosome constitution (tris 21). Isolated uncultured placental nuclei, prepared from multiple biopsies of placenta at term, were used to perform FISH with Multiprobe-I Kit (Cytocell) specific for each centromere. After FISH a case of tris 15 was detected: 84% of the isolated nuclei showed 3 signals. The standard analysis of 100 blood and 100 skin metaphases revealed a normal karyotype. UPD was excluded using 9 polymorphic loci. The histology of this placenta showed vasculopathy and the cord had 2 vessels. The delivery took place at 28th week of gestation; the weight of the baby was 540 g (< 10th centile) and after 4 months 2000 g. He showed severe micropenis, hypospadias, testes not palpable and inguinal hernia. The histology of all the other placentas shows the presence of several features: normal (11/56), chorionitis (5/56), vasculopathy (31/56), cord abnormalities (4/56), hydrops (1) and in 7 cases more than one pathology was present. UPD tests are in progress and results will be discussed.

P0963. Reproductive Decisions and Dilemas: Experience with Cordocentesis

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We present our experience in applying cordocentesis as a method of prenatal diagnosis from 1990-1999. In that period we made 674 cordocentesis, for various indications. Gravidity weeks were 18-29, and maternal age distribution was 19-43. The psychosocial characteristics of genetic advice regarding cordocentesis differ comparing to other methods of prenatal diagnosis, because fetal malformation has been already seen by ultrasound and pregnant women is “prepared” for potentially bad outcome. The other reason for different approach in genetic counseling is that termination of pregnancy is performed in high gravidity. In our work we analyzed:
- complication after the punction
- pathological finding in fetal karyotype
- follow up of the babies
- child mortality at deliverycharacteristics of genetic advice in cordocentesis and the psychosocial consequences in terminated pregnancies after cordocentesis.

P0964. Molecular-cytogenetic study of human spontaneous abortions by comparative genomic hybridization

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The frequency of spontaneous abortions is about 6-8% of all recognized pregnancies. Chromosomal aberrations are the main cause for pregnancy loss in more than 50% in the first and 20% in the second trimester of pregnancy. Conventional cytogenetic analysis of spontaneous abortions is usually used for detection of structural and numerical chromosomal aberrations, but disadvantages of the method are difficult tissue culturing and contamination by maternally derived cells. Our previous cytogenetic and molecular genetic studies have indicated that oncogenes and tumor-suppressor genes could play a role in fetal development disturbances.
Comparative genomic hybridization (CGH) is a molecular-cytogenetic technique that allows entire genome screening for numerical and unbalanced structural chromosomal aberrations in a single experiment. CGH analysis was performed on materials from 62 spontaneous abortions with aim to reveal deleted and overexpressed chromosomal regions in miscarriages.
Normal CGH profiles were found in 33 samples (53.23%). The CGH demonstrated trisomies in 20.97 % of cases (trisomies 3, 4, 5, 8, 13, 14, 14, 15, 15, 16, 16, 19, 19), monosomies in 3.23% (monosomy

X and 19), hyperdiploidy in 4 cases (6.45 %) and monosomy 9 in combination with gonosomal polysomy in one case (1.61%). Partial chromosomal gains (1p+; 1p+; 1q+; 2q+; 5p+; 5q+; 7p+; 9p+; 9q+; 10p+; 13q+; 16q+; 18p+; 22q+) or losses (16p-) were found in 9 cases (14.52%**).** In 7 out of 14 amplifications (50%) oncogenic bands were affected which is in agreement with our hypothesis of association between oncogenes and spontaneous abortions.

P0965. Mosaic trisomy 13 on chorionic villi in a fetus with body wall complex: fortuitous association or pathogenic hypothesis ?

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Body wall complex (BWC) is a malformative association characterized by the presence of abdominal wall defects associated with limb and visceral anomalies. Two distinguishable phenotypes are delineated: the first one is characterized by craniofacial defects whereas the second one includes urogenital anomalies, abdominal placental attachment and inconstant anomalies of inferior limbs.
We describe here a fetus with BWC detected by ultrasound at 16 weeks' gestation. Direct chromosome analysis of chorionic villi displayed a trisomy 13 in 20 of 21 cells whereas the cultured cells showed a normal karyotype 46,XY. Foetopathological examination demonstrated a male 17-week-old fetus with an inferior celosomy associated with a short umbilical cord, urogenital anomalies and limb defects. Postmortem karyotype from skin fibroblasts was normal.
Little is known about the pathogenic mechanisms of BWC. At present, the three main theories are early amnion rupture sequence including all phenotypes of BWC, early vascular disruption involved in the craniofacial phenotype and early embryonic maldevelopment with disturbance of the embryonic folding at the origin of the second phenotype. The latter could result of a malfunction of the ectodermal placode or rather of a disturbance of the ectodermal cells deposition into the mesodermal compartment, as suggested by Hartwig (1992).
To our knowledge, chromosome anomalies have not been reported in BWC. Although our results may be fortuitous, it is striking to observe a discrepancy between direct cytogenetic analysis of ectodermal cytotrophoblastic cells and cytogenetic analysis of cultured extra-embryonic mesodermal cells when BWC might result of abnormal ectodermal and mesodermal interactions.

P0966. Prenatal diagnosis of a diploid/tetraploid fetus

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Tetraploidy is characterized by four complete sets of chromosomes (4n = 92). Although it has frequently been reported in early spontaneous abortions, tetraploidy is extremely rare in term pregnancy. Most of late surviving patients are diploid/tetraploid mosaics and present severe mental and physical impairment. To our knowledge, only four tetraploidies were ascertained in the prenatal stage on amniocytes and/or fetal blood lymphocytes.
We report here on the prenatal diagnosis of a diploid/tetraploid fetus: at 11 weeks' gestation, a karyotype performed on chorionic villi (direct analysis and cultures) for cystic hygroma showed full tetraploidy. Subsequent amniocentesis was considered as normal (despite the presence of a tetraploid clone among the 15 analysed). However a sonographic examination at 18 weeks' gestation displayed a complex cardiopathy. After termination of pregnancy, chromosome analysis on different tissues confirmed diploid/tetraploid mosaicism.
This observation and the four previously reported in the literature emphasize the difficulty of the prenatal diagnosis of true tetraploidy, first because the features are not specific, often mild or revealed late in pregnancy, second because tetraploidy may be an artifact on in vitro cell cultures or a confined placental mosaicism.
Therefore, in a context of ultrasound abnormalities, when cytogenetic analysis displays tetraploidy, caution is advised and karyotypes on other tissue samples have to be performed and compared with control cultures. Thus the distinction between artifactual and true tetraploidy will be possible.

P0967. The prospective analysis of prenatal diagnosis of aneuploidy in Croatia

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The great majority of chromosomal abnormalities (approximatelly 95%) are due to numerical variation of chromosomes 13, 18, 21, X and Y. For that reason, prenatal diagnostics is necessary to follow up all pregnancies at risk. During 1978-2001, amniocentesis was performed on 17 004 pregnant women. There were following indications for prenatal diagnosis; 72% maternal age of 35 and higher, 4.9% previous child with Down syndrome, 4.74% medicine, X-ray and viruses, 2.12% ultrasound identification of fetal anomaly, 1.81 malformations in children, 1.5% other chromosomal abberations, 1.06% positive maternal serum screening, 1.17% autosomal genopaty, 0.2% history of structural chromosome abnormality in one of the parents.There were found 358 (2.11%) of chromosomal anomalies. Among them 298 (1.75%) are aneuploidies. Results are shown in table.

INDICATION	Trisomy 21	trisomy 18	trisomy 13	sex CHROMO. aneuploidy	TOTAL
Maternal age	150	39	9	37	235
Previous child with Down syndrome	4	1		2	7
Ultrasound identification of fetal anomaly	8	10		6	24
Malformations in children	4		1	5	10
Other chromosomal aberrations	1	2		4?	7
Medicine, X-ray, viruses				7	7
Autosomal genopaty				1	1
Num. abberations in previous pregnancies		1		3	4
Psyche				3	3
TOTAL	167	53	10	68	

In conclusion data of this analysis are in concordance with results from other studies. Maternal age is one of the primary indications for prenatal diagnosis of aneuploidy and trisomy 21 is the most common aneuploid condition compatible with survival.

P0968. The evaluation the effectiveness of nuchal translucency measurement in screening for congenital heart disease

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OBJECTIVES: To evaluate possibility first trimester marker, used for Down syndrome screening, named nuchal translucency (NT),for congenital heart defect diagnostics.
METHODS: An unselected group of 3003 pregnant women with a singleton pregnancy underwent first trimester screening at 10-13 weeks' gestation in 2000-2001. Nuchal translucency (NT) was measured by transvaginal sonography. Invasive procedures for fetal karyotyping in cases with NT > or =3 mm were performed and fetuses with abnormal karyotypes were eliminated. A second trimester detailed ultrasound scan was also performed in all cases with nuchal translucency thickness. We tried list of all registered cases of congenital heart defect in newborns and babies from neonatologists, pediatricians, heart surgeons and other sources.
RESULTS: There were 84 cases with NT>3 mm. In this group was found 17 cases of chromosomal anomalies (21+, 45X, 18+). Twelve pregnancies were lost to follow-up .In only 1 case we found two chamber heart. Other outcomes were normal
CONCLUSION: First trimester nuchal translucency measurement is an effective screening test for the prenatal detection of fetuses with

chromosome anomalies, including Down's and Turner syndromes, but effectiveness in congenital heart defect finding requires further study.

P0969. Infertile couples and prenatal cytogenetic diagnosis

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From 17 004 prenatal cytogenetic investigations, 889 pregnant women have had an indication for early amniocentesis because of infertility. Chromosome analysis of cultured amniocytes with GTG banding showed 13 (1,46%) unbalanced and (2,92%) balanced karyotypes. Thirteen cases of pathological karyotype include 7 trisomies, 4 numerical aberrations of gonosomes and 2 unbalanced translocations , and 10 among them were over the age of 35, that increased the risk.From 26 balanced karyotypes, reciprocal translocation were more common (17; 65.4%) then the robertsonian type (9; 34.6%). A total of 889 infertile couples, in 51 cases one of the parents was already known as a carrier balanced rearrangement.In 20 fetal karyotypes (2.25%) was detected that one of the parents was balanced translocation carrier.It was found one case of de novo robertsonian translocation. The analysis shows high excess of female (20) over male (6) carriers.Prenatal cytogenetic diagnosis should be performed in pregnancies of infertile couples who have had two or more pregnancy losses. They are at risk for carrying a balanced rearrangement since these carriers may lead to offspring with an unbalanced karyotype causing serious congenital anomalies. Many chromosomally imbalanced fetuses are spontaneously aborted before amniocentesis, but partial trisomies of small rearrangements tend to be maintained in pregnancy. Because of the high incidence of chromosome abnormalities in spontaneous abortions at infertile couples, prenatal diagnosis is needed as well.

P0970. The significance of congenital anomalies to gestational wastage in a Brazilian University Hospital

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It is described a prospective investigation at the Women's Hospital (Caism), to determine the precise causes of gestational wastage. This study included all perinatal deaths (PD) occurring in the obstetrical center at the Hospital between Sept/1999 to April/2001. All PD were analysed with a protocol including genetic-clinical examination, X-rays, clinical photographs, autopsy, and cytogenetic investigation when necessary. From a total of 228 PD, 80 fetus (35%) were malformed. The causes of gestational wastage were classified as maternal (85 cases, 37%), fetal (104 cases, 45%), or unknown (39 cases, 17%). Fetal causes were more commonly found in the group of early neonatal deaths. Besides obstetrical causes, the other main diagnosis found among PD from maternal origin, were arterial hypertension and infectious diseases. Among PD from fetal causes, 24 cases (23%) were twins. The remaining were all malformed foetus distributed as follows: 35 (44%) isolated defects, 15 (19%) foetus with multiple anomalies, and 30 (37%) syndromic fetus. In the isolated group congenital malformations of the CNS (22) followed by uro-genital (12) were the most frequent anomalies. Chromosomal and disruption syndromes, besides skeletal dysplasias were the main syndromes diagnosed. With regard to all the known causes of deaths in the whole group, we observed that secondary prevention is possible in more than half of the deaths with maternal origin. Among deaths from congenital anomalies of the foetus, prevention by genetic counselling and/or prenatal diagnosis is possible in more than 80% of the cases.

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P0971. Rapid prenatal diagnosis of common autosomal aneuploidies by relative semi-quantitative fluorescence PCR on uncultured amniocytes

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Prenatal diagnosis of chromosomal abnormalities by cytogenetic analysis is time consuming, expensive and requires highly qualified technicians. Rapid diagnosis of aneuploidies followed by reassurance for women with normal results can be performed by molecular analysis of uncultured foetal cells in less than 24 hours. Today, all molecular techniques developed for a fast diagnosis of aneuploidies rely on the semi-quantification of fluorescent PCR products from short tandem repeat (STR) polymorphic markers. Our objective was to test a chromosome quantification method based on the analysis of fluorescent PCR products derived from non polymorphic target genes. An easy to set up co-amplification of portions of DSCR1 (Down Syndrome Critical Region 1), DCC (Deleted in Colorectal Carcinoma), and RB1 (Retinoblastoma 1) allowed the molecular detection of aneuploidies for chromosomes 21, 18, and 13 respectively. Semi-quantitative analysis was performed in a blind prospective study of 400 amniotic fluids. Four samples (1%) could not be analysed by PCR probably because of a low concentration of foetal DNA. Follow up karyotype analysis was done on all samples and molecular results were in agreement with the cytogenetic data with no false-positive or false-negative results. Our gene based fluorescent PCR approach is an alternative molecular method for a rapid and reliable detection of aneuploidies which can be helpful for the clinical management of high-risk pregnancies.

P0972. Deletion 22q11 and conotruncal cardiopathy in four successive pregnancies: contribution of prenatal diagnosis.

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CATCH 22, an acronym for cardiac defects, abnormal facies, thymic hypoplasia, cleft palate, and hypocalcemia is associated with a variable deletion on chromosome 22q11 and occurs in patients with dysmorphologic and cardiological syndromes: DiGeorge, velocardiofacial and conotruncal/face syndrome. Estimates suggest that the 22q11.2 deletion occurs in approximately 1/4000 live births, making this disorder a significant health concern, so much so that 22q11.2 deletion studies are becoming part of a standardized diagnostic workup for some isolated heart defects. We report on 4 consecutive 22q11.2 deletion antenatal diagnoses, in an asymptomatic 22q11.2 deletion carrier, ascertained following in utero detection of a conotruncal cardiac defect. Her first pregnancy resulted in a normal female. The second and 3rd pregnancies were terminated following ultrasonographic visualization of conotruncal cardiac defect. FISH analysis of amniotic fluid from the second affected pregnancy confirmed the diagnosis of 22q11.2 deletion, for which the mother herself tested positive. FISH performed on CVS from her 4th and 5th pregnancies were again consistent with the diagnosis of 22q11.2 deletion. Cardiac defects detected on ultrasonograph of the 4th pregnancy were not visualized during the 5th pregnancy, which was terminated on week 15. The ethical issue imposed by termination of what seems to be a normal fetus, albeit carrying 22q11.2 deletion, is underlined. This report highlights the importance of offering 22q11.2 deletion testing to parents of affected probands and couples following in utero detection of a cardiac defect. Antenatal knowledge of the deletion status provides couples with an accurate diagnosis, prognostic information, and recurrence risk.

P0973. Three cases of deletion 13q

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Abstract

Three cases of deletion 13q

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We present three cases of deletion in the long arm of chromosome 13 discovered in routine amniocentesis because of maternal age and one case with ultrasonografic indication.

Cases 1 and 2 show a structural aberrant karyotype, with breakpoint in 13q31.

We compare ultrasonografic findings, which showed severe fetal abnormalities at the cerebral features, like holoprosencephalie and exencephalie.

The last one is a special case. Here we present a ring chromosome with deletion in the long arm of chromosome 13. The deleted region of the ring chromosome is established by F-PCR (ABI 310).

P0974. Alpha1-antitrypsin deficiency due to maternal uniparental disomy for chromosome 14

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Alpha1-antitrypsin is a major inhibitor of serine protease and plays a crucial role in protecting pulmonary tissue from degradation by neutrophil elastase. Alpha1-antitrypsin deficiency, an autosomal recessive disorder, predisposes individuals to the development of pulmonary emphysema and is also associated with chronic liver disease. The PI gene is located on the long arm of chromosome 14 at position 14q32.1. Numerous normal or deficient variants of this gene have been described but two major mutations (Z and S) are responsible for the pathology.

We report on a child with developmental delay and alpha1-antitrypsin deficiency (ZZ). His mother is heterozygous for the Z mutation but his father presented with a normal genotype (MM).Exclusion of paternity was discarded and then we began a molecular analysis to explain the genotype discordance and the particular phenotype of the child. Six microsatellites on the long arm of chromosome 14 (D14S264, D14S1057, D14S258, D14S76, D14S67, D14S1162) were tested. Analysis of DNA polymorphisms shows no contribution of the father and two chromosomes 14 from the mother with isodisomy for markers near the PI locus and heterodisomy for the more centromeric markers.

Uniparental disomy as a mecanism of recessive disorders may be evocated when the implicated chromosomic region carries a mutated gene.So, cystic fibrosis due to disomy of chromosome 7 has been reported. We report here the first case of alpha1-antitrypsin deficiency due to disomy of chromosome 14.

P0975. Prenatal diagnosis of congenital lipoid adrenal hyperplasia (CLAH) by measuring maternal serum unconjugated estriol(MSuE3) in the 1st trimester of pregnancy.

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CLAH is a rare autosomal recessive disease, quite common in the Palestinian population in Israel and is usually diagnosed after birth. The clinical picture is adrenal insufficiency at birth: dark skin, vomiting and failure to thrive. The phenotype is of a female although the karyotype is XY. Unless treated early, death can occur shortly after birth. Treatment with steroids and salts is required for lifetime. The basic defect in this disease is no production of any steroid: cortico-mineralo- and sex steroids. The genetic defect is in the StAR gene, encoding a cholesterol shuttle protein, which transports cholesterol into the mitochondria, where the process of steroidogenesis takes place.

Prenatal diagnosis of CLAH is possible by mutation analysis of the StAR gene, or measuring the MSuE3 during 1st trimester of pregnancy, between 10-13 weeks. We will present our experience in diagnosing fetuses with CLAH and other types of fetal adrenal insufficiency in the 1st trimester of pregnancy. We are using a non-invasive method, which, to our best of our knowledge, has not been offered before at such early stage of pregnancy.

P0976. Somatic microsatellite instability in prenatal samples detected by QF-PCR

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Mosaicism (the presence of two or more different cell lines) is reported to occur in approximately 1 % of chorionic villus samples (CVS), although one cell line may be confined to the placenta. Rapid aneuploidy testing is now being applied in many cytogenetic laboratories either to complement or replace conventional karyotype analysis. Assessment of these technologies includes their ability to detect potentially significant abnormalities such as mosaicism. Quantitative fluorescence-PCR (QF-PCR) detects genomic imbalance by comparing the alleles of amplified STR markers. Routine use of QF-PCR in our centre (>3000 samples to date) has established that this technology can detect low level trisomy mosaicism. However, a previously unreported form of mosaicism at the molecular level was also observed. Nine out of 275 (3.3%) CVS samples showed frond-specific de novo STR alleles. In two of these samples instability was confirmed (one 8bp expansion of D13S742 and one 4bp expansion of D21S1411) by investigation of parental DNA, and in seven by the absence of the novel allele from other fronds and from cultured cells. Mosaicism for a novel allele at a single locus was also detected in uncultured material and cultured cells from one amniotic fluid sample. Eight different STR markers to date have demonstrated novel alleles. Although repeat instability is unlikely to represent an abnormal phenotype, the relatively high frequency of somatic mutation in individual villi is surprising. Case results, possible mechanisms and implications for the interpretation of QF-PCR results will be discussed.

P0977. Triploidy/tetraploidy placental mosaicism: a new mechanism of foetal triploidy

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Triploidy and tetraploidy are detected in 20% of early spontaneous abortions and a few fœtuses survive beyond midgestation. Polyploid mosaicism is very uncommon. We report seven spontaneous abortions with triploid/tetraploid placental mosaicism, with four empty sacs at seven to sixteen weeks of gestation (WG) and three cases with placenta and fœtus at sixteen, seventeen and twenty-two WG. Karyotyping was not available.

DNA quantification was carried out after pathological investigation suggestive of triploidy The two oldest fœtuses had severe growth retardation (-3SD), syndactyly, adrenal hypopoplasia. The histology of the seven placentas showed hydropic villi with cisterns, vessels, trophoblast hyperplasia and scalloping of the fibrous villi. A few villi exhibited enlarged nuclei. Paraffin embedded placenta sections were used for DNA image cytometry after Feulgen staining, using the CAS 200 image analysis system. At least 200 nuclei were analysed in the mesenchyme of the villi and maternal decidua was used as control (diploid cells with DNA index [DI] near 1). In the seven placentas DI was near 1.5 (triploid nuclei) in 36 to 59% of the cells and near 2 (tetraploid nuclei) in 38 to 51% of the cells. The two oldest fœtuses were triploid (DI near 1.5).

Abnormal fertilization with diandry or digyny are the usual mechanisms reported in triploidy. The seven cases of triploid/tetraploid mosaicism give a new insight with a third mechanism taking place later during segmentation. It could be due to abnormal development of the poles of the spindles during mitosis.

P0978. Pregnancy outcomes in women with CPM: 12 cases report.

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Confined placental mosaicism (CPM) is reported to be associated with adverse pregnancy outcomes in form of spontaneous abortions, preterm births and intrauterine growth retardation. Pregnancy progression and perinatal complications in 12 patients with CPM, detected in the first-trimester CVS, were studied. Chromosomes, involved in mosaicism in cytotrophoblast, were different, involving 3,7,14,19,21 and X . Average age of pregnant women was 33,7±7,2 years (M±SD), 7 patients were 35 years and older. The mean value of maternal serum alpha-fetoprotein was 1,33±0,56 MoM and maternal serum chorionic gonadotropin (MShCG) 2,49±1,41 MoM, abnormal high level of MShCG (>2,0 MoM) was noticed in 5 patients. Uterine artery Doppler velocimetry during pregnancy was performed with 4-weeks intervals. Average uterine systolic-diastolic ratio and pulsatility index was found elevated in women with CPM compared with control group at 20 (S/D =2,96±0,23 and 2,13±0,05; p<0,001) and 36 weeks of pregnancy (S/D =2,32±0,17 and 1,71±0,05; p<0,001). Pregnancy complications and adverse outcome were registered in 2/3 of women with CPM. Preeclampsia developed in 3 patients. Stillbirth was registered in 4 cases (1 case of spontaneous abortion at 26 weeks and 3cases of preterm delivery before 37 weeks of pregnancy); intrauterine growth retardation was detected in 3 cases. Morphological signs of the placental insufficiency and villi

immaturity were observed in all placentas. Our data reflect a possible negative effect of CPM on the pregnancy outcome, probably due to the placental abnormalities, and supports the previous reports of association between CPM and adverse pregnancy outcome.

P0979. Rapid X chromosome dosage by Quantitative fluorescent Polymerase Chain Reaction (QF-PCR) and prenatal diagnosis of Turner syndrome

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The quantitative fluorescent polymerase chain reaction (QF-PCR) is an assay designed to perform rapid prenatal detection of common numerical chromosome abnormalities. This method is based on the PCR amplification of highly polymorphic short tandem repeats (STRs). In the course of PCR amplification, a fluorochrome is incorporated into the products, which are then visualised and quantified using an automated DNA sequencer. Several investigations have documented the accuracy of performing rapid prenatal diagnoses of trisomies involving chromosomes 21, 18 and 13.

However, due the unavailability of highly specific STR markers, only in recent times it has been possible to detect X and Y chromosome abnormalities. For the detection of Turner syndrome, several X-linked STRs are included in multiplex PCR assays so that, using up to 4 markers, the likelihood of a normal female to be homozygous for all sequences, is expected to be extremely low (about 2/1000).

Here we report a new method for rapid detection of X chromosome copy number in all prenatal samples. The test is based on QF-PCR amplification of the X-linked HPRT with the autosomal D21S1411 used as internal control for quantification. X chromosome copy number is rapidly and accurately assessed by comparing the ratio between the fluorescent activity of the X specific and autosomal PCR products, thus independently from any calculation of probability.

In its first clinical application this method allowed distinguishing a rare normal female foetus, homozygous for all X chromosome markers used, from a Turner syndrome.

P0980. Application of fluorescence in situ hybridization in prenatal diagnosis for identification of rare structural aberration.

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We present results of cytogenetic prenatal investigations in 5 cases of de novo chromosomal rearrangments impossible to diagnose with routine cytogenetic banding techniques. Fluorescense in situ hybridization (FISH) with multiple chromosome specific libraries (chromosome painting) and subtelomeric Chromoprobe Multiprobe T System (Cytocell) were applied to identify or verify these rearrangments.

In two cases translocated fragments were so small, that we had to apply FISH to find out whether the translocations were balanced. In one case we found a small additional fragment on the short arm of chromosome 15. FISH enabled us to identify it as a part of chromosome 2. It coincided with inversion of chromosome 10 in the father. In one case we found de novo pericentric inversion of chromosome 3. In the last case the kariotype was mosaic 45,XO/46,X+mar. In amniocytes the marker was present in 28% of the cells, in leucocytes (obtained by cordocentesis) - in 90 % of the cells. Using painting and subtelomeric probes we found that the marker contained sequences of Y. We also found p-arm subtelomeric region of Y or X chromosome (cohybridization X and Y p-arm) on both ends of Y. Ultrasonography has shown that the fetal sex was male.

P0981. Application of fluorescence in situ hybridization to chromosome analysis in prenatal diagnosis

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For over two decades banding has remained the “gold standard” of cytogenetic analysis, providing the first genome-wide screen for abnormalities. However, these analyses are complemented with techniques based on fluorescence in situ hybridization-FISH, which steadily increased the accuracy of cytogenetic diagnosis. The basis of in situ hybridization techniques is the detection of specific nucleic acid sequences on cytogenetic level.

In our laboratory FISH is one of the routine methods of prenatal diagnosis, which previously was introduced and optimized, for molecular cytogenetic diagnostics in oncohematological disorders of over 50 cases. In this study it was applied for detection of trisomy 21 in 11 cases and also for searching of some aberrations of sex chromosomes. Nuclei were prepared from a culture of chorionic villi in three cases and amniotic fluid in the others. Hybridization was performed with specific X/Y and 21q22 probes, and detected with FITC and spectrum orange. In one sample of all analyzed interphase nuclei were found three signals after hybridization with 21q22 probe, indicating that three chromosomes 21 exist (Down syndrome). After using X/Y probe in one case was detected monosomy X, without signal for chromosome Y (Turner Syndrome).

Except for the accuracy of the results, with routine implementation of FISH into laboratories a lot of obstacles, like cases of advanced pregnancy, or problems with cultivating of cells could be overcome. FISH is now the complementary method of choice because of the increased sensitivity and speed with which it can be applied to a variety of cellular targets.

P0982. The mutational and haplotype analysis of the phenylalanine hydroxylase gene in families with phenylketonuria from the Bashkortostan.

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Phenylketonuria (PKU) is a common autosomal recessive genetic disorder caused by a large variety of mutations in the phenylalanine hydroxylase (PAH) gene.

This study repots the results of mutational and haplotype analysis in 56 families with phenylketonuria from the Bashkortostan. Our results indicate that the R408W mutation account for over 53% PKU in Bashkortostan. Using SSCP analysis followed by sequencing of 7 and 12 exons of the PAH gene we have identified 5 mutations: R261Q (9,8%), R252W (2,7%), P281L (2,7%), R252P (1%) and 1315+del4 (1%).

We have examined the distribution of haplotypes of four polymorphic alleles of the PAH gene (VNTR, MspI(a), STR and PvuII(a) alleles). Most common PAH haplotype (380-MspI(a)a-240-PvuII(a)A2) appeared in 47% of the PKU chromosomes, but rare occurred on normal chromosomes (4%).

Moreover, we have studied haplotypes associated with PKU mutations. The most prevalent PAH haplotype 380-MspI(a)a-240-PvuII(a)A2 was tightly linked to the most common R408W mutation (67%) and the R261Q mutation (82%). The others mutations detected in this study were associated with various haplotypes.

Thus our investigations demonstrated a strong correlation between the R408W mutation and PAH haplotype 380-MspI(a)a-240-PvuII(a)A2, which correspondents to RFLP-haplotype 2. These dates suggest a common origin for this mutation from Europen populations, where R408W mutation is strongly associated with haplotype 2.

In addition, we have determined the informativity of PAH haplotype for molecular diagnostics of PKU in Bashkortostan that was 95,5%. The data of haplotype and mutational analysis may be used for prenatal diagnostics and carrier screening in PKU families.

P0983. Trends in cytogenetic prenatal diagnosis in a reference hospital in Izmir/Turkey: A comperative study for 4 years

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The aim of the study was to investigate the major changes in the indications, culture success and abnormality rate for conventional cytogenetic prenatal diagnosis for amniotic fluid samples between the period of January 1998 and December 2001 in our area.

Our cytogenetic laboratory provides a prenatal service to obstetrics-gynecology departments of different hospitals in Izmir. Limited

number of patients (6-8 per week) is randomly accepted for prenatal cytogenetic study in our center.
Over the 4 years period 1023 prenatal cytogenetic tests were performed in our center. The most common indication was advanced maternal age for each year. But its rate has increased significantly within the years. Culture success rates have improved. When the first two years compared to the last two years the rate of abnormal cytogenetic results were significantly decreased.
The major reason for this observation is probably related to the changes in indications throughout the years.

P0984. Seven cases of chromosomal mosaicism detected in amniocentesis and karyotype, phenotype correlations.

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Cytogenetic test results of 908 amniocentesis performed throughout three years period were evaluated.
Out of 908 amniocentesis, seven cases (0.77%) were detected having the chromosomal abnormalities in more than three cells in total. In all of these cases, different chromosomal variations were observed in abnormal cell lines.
Out of seven cases, four of them were detected with numerical chromosomal abnormalities in mosaic cell line. These were 46,XY[110]/47,XXY[11]; 46,XY[96]/47,XY,+21[4]; 46,XY64]/47,XY,+22[12]; 46,XY[35]/47,XYY[15]. The remaining three cases had structural chromosomal abnormalities in mosaic cell lines. Two of these were balanced translocations (46,XY[150]/46,XY,t(2;12)(q23;q24.2)[5]; 46,XY[65]/46,XY,t(7;8)(q22;p23)[5]) whereas the other one was a rarely seen chromosomal deletion (46,XX[95]/46,XX del 18(q12.1-qter)[5].
In all of the three cases with mosaic structural chromosomal abnormalities fetal cord blood showed normal fetal karyotype and the outcome of these pregnancies were also normal.
Only in one of the four cases with mosaic numerical abnormalities, mosaicism was also detected in the fetal blood and the family chose abortion. The others' karyotypes in fetal blood were normal (and one of these resulted in spontaneous abortion while the other two resulted in normal outcome).

P0985. Prognostic Dilemma in Genetic Counseling of Prenatal Cytogenetic field

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Objective; With an increasing number of pregnancies being subjected to prenatal karyotyping, large proportions of fetuses with borderline prognosis are detected prenatally as an incidental finding. The couples are faced with a very difficult and personal decision for the pregnancy. This study was designed to evaluate prenatal outcome of borderline prognostic cases in prenatal cytogenetic field. **Materials and Methods;** We reviewed retrospectively pregnancy outcome of 9,002 prenatal cytogenesis cases during 1990 - 2000. We classified with 4 groups according to prognosis. For precise determination of results, several cytogenetic methods were performed; repeat sampling, C-banding, R-banding, FISH, CGH, NOR banding and so on. **Results;** Group I (normal); (N=8661, 96.2%), Group II (balanced cases from parental origin); (N=87, 0.97%), Group III (apparently abnormal cases); (N=211, 2.35%, Group IV (borderline cases); (N=43, 0.48%). Through several cytogenetic studies, targeted fetal ultrasound and genetic counseling for borderline cases, pregnancies were continued and delivered healthy babies in 3 cases out of 6 de novo balanced reciprocal translocation, 2 out of 3 de novo robertsonian translocation, 15 out of 25 sex chromosomal aneuploidy, 3 out of 6 marker chromosome, 3 out of 3 discrepant results with prenatal samples. **Conclusions;** For genetic counseling of borderline cases, molecular cytogenetic study and targeted fetal ultrasound played an important role for option of pregnancy continuation.

P0986. Efficiency of Dyna-beads methods for non-invasive prenatal genetic diagnosis using fetal cells

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Prenatal genetic diagnosis has become an essential part of modern obstetric care. Recent advancements in molecular technology and equipments have made fetal diagnosis possible. During the last two decades a number of methods of prenatal genetic diagnosis have become available and have used either in laboratory research or in routine genetic counseling. Non-invasive prenatal genetic diagnosis by circulatory fetal cells in maternal circulation also taking shape. The advantage of non-invasive methods (fetal cells and DNA in maternal blood) is that they provide an opportunity to make a genetic diagnosis without risk therefore, are applicable for use in mass screening programs. We obtained approximately 20 ml blood from 53 women. The mononuclear cells were isolated with ficole. To remove unwanted maternal white blood cells, antibodies against CD45 and CD14 coated with magnetic beads were used as negative selection. Positive selection methods based on using antibody against CD71 and Dynal magnetic to enrich fetal nucleated erythrocytes. DNA was extracted from fetal cells by a standard phenol-chloroform procedure and nested PCR was carried out with appropriate primers for RhD gene and amelogenine gene was used for sex (X and Y) determination. These results suggest that identification of fetal sex and RhD in the maternal blood circulation in 27 or %51 of the case is possible but efficiency of this method compare to others is low.

P0987. Evaluation Of A Multiplex QF-PCR Assay For Aneuploidy Testing

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Short tandem repeats (STRs) are highly informative polymorphic genetic markers, widely used in the field of quantitative fluorescent PCR (QF-PCR) aneuploidy testing. With the intention to reduce pipetting errors, cost value and time for analysis, we have developed a multiplex PCR assay in a one-tube reaction for simultaneous analysis of two STRs located on chromosome 21 (D21S11 and D21S1411), one - located on chromosome 18 (D18S535) and one - on sex chromosomes (AMEL). PCR amplification with four Cy-5' fluorescently-labeled primer sets was performed and PCR products were separated on ALFExpress sequencer. Because of inability to use different fluorescent dyes, STRs were selected with different PCR product lenghts, with reported heterozygosity between 0.90 and 0.93. To avoid preferential amplification of shorter fragments and to receive the expected allele ratio the optimal primer amount was adjusted between 2-5 pmol of each primer, number of cylces = 25. The multiplex PCR was optimised with human genomic DNA of normal controls and trisomic 21 samples, no unspecific products were observed. Reported four-plex PCR assay proved to be rapid, accurate and efficient - results were in complete accordance with those obtained after performance of 4 separated single-plex reactions among the same 65 investigated DNA samples.

P0988. Applications of two-dimensional electrophoresis and fluorometric enzyme assay for MPS prenatal diagnosis

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The mucopolysaccharidoses (MPS), a group of heritable disorders, are transmitted in an autosomal recessive manner, except for MPS II(X-linked). The diagnosis of MPS can be achieved by two-dimensional electrophoresis (2-D EP) for MPS typing determination and enzymatic assay for MPS confirmation. In 2000 - 2001, we had

successfully completed prenatal detection by 2-D EP and fluorometric enzyme assay in seven unrelated families having previous affected children; 5 with MPS II, 1 with MPS III and 1 with MPS IV. Amniotic fluids (AF) were taken at 14th ~ 18th weeks of gestation. For 5 AF with high risk of MPS II, the results showed normal EP pattern, in which the chondroitin sulfate (CS) and hyaluronic acid (HA) were demonstrated. The activities of iduronate-2-sulfatase (IDS) were normal in the cultured AF cells. For pregnancy with high risk of MPS III, the 2-D EP result showed a composed pattern of CS, HA, and heparan sulfate (HS). For pregnancy with high risk of MPS IV, the result showed a composed pattern of CS, HA, and keratan sulfate (HS). The enzyme activities of alpha-N-acetyl glucosaminidase and galactosamine 6-sulfatase were all reduced in the cultured AF cells, which were corresponding with the 2-D EP results. On the basis of these results the pregnancies were terminated. By reviewing the 2-D EP method, it is definite, sensitive, and easy to perform for MPS prenatal diagnosis, however, a final confirmation is required by performing an enzymatic assay of that specific enzyme activity.

P0989. Characterisation of the gene defect in 12 patients with sporadic haemophilia B.

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Previous studies have shown that haemophilia B (HB) is the result of several mutations, mostly single nucleotide substitutions, in the Factor IX gene. In order to evaluate the impact of mutation analysis on genetic counselling in sporadic and uninformative HB familial pedigrees, we re-analysed 12 patients by dHPLC, who had previously been studied by restriction fragment length polymorphisms (RFLPs). In order to perform mutation analysis, the coding regions, intron/exon splice junctions , 5' and 3' untranslated regions, and the promoter and polyadenylation site of Factor IX gene were amplified using the appropriate primers. In all cases unique abnormal dHPLC chromatograms were found, the specific abnormal fragments were sequenced and the mutations were characterised. The distribution detected in our screening studies fits with what is reported in the FIX mutation database. 93% of the mutations occurred in the coding sequences of the gene. They were all independent mutations, mostly (9/12) associated to the severe form of the disease. Point mutations were the most recurrent mutations. Three mutations were novel, unreported in the HB database and none involved CpG dinucleotides. Ten mutations were single base substitutions, one was a base insertion, and one was a four nucleotide deletion. By identifying the detrimental mutations in affected males, carrier status was correctly diagnosed in all the women we studied. 3/12 de novo events were found in maternal meioses.

P0990. Discordant prenatal diagnosis due to a mosaic structural rearrangement of chromosome 21 in two trisomic 21 fetuses.

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Trisomy 21 mosaicism associated with a structural rearrangement is uncommon. We report on two prenatal diagnoses in which karyotypes showed mosaicism with an aberrant cell line including a rearrangement of chromosome 21. Prenatal diagnoses were performed because of increased nuchal translucency. In the first case, analysis of trophoblast cells revealed an abnormal karyotype: 46,XX/47,XX,+del(21)(q21). The amniocentesis and cordocentesis showed a non-mosaic trisomy 21. In the second case, the trophoblast direct analysis showed a normal male karyotype whereas the long-term culture revealed trisomy 21 mosaicism secondary to a rearrangement of chromosome 21 (either a Robertsonian translocation or an isochromosome): 46,XY/47,XY,rea(21q21q)/45,XY,-21. Amniocentesis confirmed the trisomy 21 in all cells. FISH analysis proved that these rearrangements were derived from chromosome 21. The mechanisms of formation are discussed. The first case could be explained by the deletion of one chromosome 21 in a trisomic zygote with instability of this de novo marker leading to a normal cell line. In the second case different mechanisms are suggested. The rearrangement might occurred postzygotically in a normal embryo with a non-disjunction or a translocation of homologous chromatides of one chromosome 21 leading to a cell line with the rearranged chromosome 21 and another cell line with a monosomy 21. A more complex mechanism of formation is also considered with a cascade of meiotic and subsequent mitotic errors. Our cases underline the importance to combine the short-term and long-term cultures and emphasises the need for confirmatory studies in other tissues when mosaicism is encountered in chorionic villi.

P0991. Molecular Diagnostics Of Bulgarian Patients With 17p11.2 Duplication/ Deletion Using Two Sets Of Highly Polymorphic DNA Markers

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Charcot-Marie-Tooth (CMT) disease is the most common inherited disorder of the peripheral nervous system. The majority of patients with demyelinating neuropathy (CMT1) have 1,5Mb duplication/deletion in 17p11.2 chromosome region. It is responsible for nearly 80% of authosomal-dominant CMT cases.
We have analyzed a total of 28 CMT1 families with clinical data for authosomal-dominant inheritance for 6 highly polymorphic markers closely linked to 17p11.2 region: D17S921, D17S122, D17S1357, D17S1358, D17S2226 and D17S2227. We have set up analytical conditions on ALF fragment analyzer to receive reliable and reproducible gene dosage differences in affected subjects and controls. We have detected 11 families with duplication and 1 with deletion. The combination of markers D17S921 and D17S122 was informative in 9 cases. Additionally testing for D17S1357, D17S2227 and D17S2226 revealed another 3 cases of 17p11.2 duplication. With these five markers we were able to identify duplicated/deleted and normal alleles in 100% of CMT1A and HNPP families. Polymorphic markers have been arranged in two sets based on their informativeness and length of repeats. The first one included D17S921, D17S122, D17S2227 and allowed correct and fast molecular diagnosis of 17p11.2 duplication/deletion in 90% of cases. The second one comprising D17S1357and D17S2226, we used in non-informative cases and as a verification test. Marker D17S1358 was informative in only 2 cases and therefore it is not currently used in our laboratory diagnostic practice. The described two-step procedure is fast, efficient and inexpensive, which makes it suitable for routine postnatal and prenatal diagnostics of 17p11.2 duplication/deletion in Bulgarian CMT1 families.

P0992. Recurrence of achondrogenesis type II within the same family: evidence for germline mosaicism

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Achondrogenesis type II is a lethal skeletal dysplasia, characterized i) clinically by short-limbed dwarfism with short trunk, anasarca, and disproportionately large cranium, and ii) radiologically by lack of complete ossification of the vertebral bodies. New dominant mutations within *COL2A1* have been identified in this disorder. Here we report on two pregnancies of a healthy, nonconsanguineous young couple. In the first pregnancy, severe micromelia and generalized edema were noted on ultrasound at 21 WG. Clinical evaluation of the fetus after termination of the pregnancy revealed short-limbed dwarfism with short trunk, narrow thorax, large head, generalized edema, flat face, short nose with anteverted nostrils, micrognathia and short neck. Radiographs showed typical findings of achondrogenesis type II, including very short and squared tubular bones, with irregular metaphyseal spurring, short iliac wings and absence of ossification of ischiatic and pubic bones. The vertebral bodies were very poorly ossified and bipartite in the dorsal

region. Metacarpals and phalanges were small, with punctiform first metacarpals. Histologic studies confirmed the diagnosis. In the second pregnancy, fetal hygroma was noted at 11 WG and similar clinical, radiographic and histologic findings were observed. Molecular analysis of gDNA extracted from amniotic cells of the second fetus revealed heterozygosity for a G316D mutation in the COL2A1 gene. This mutation was absent in gDNA extracted from the parental blood lymphocytes. Although we could not prove the presence of the mutation in the first fetus because of lack of appropriate materials, we strongly believe that our data are in favour of germline mosaicism.

P0993. Prenatal diagnosis of partial trisomy 18 by quantitative fluorescent PCR (QFPCR)

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The aim of this report is to document the importance of the QFPCR for rapid prenatal diagnosis. This pregnancy was referred in 24th week because of decreased maternal serum hCG (0,23 MoM) and abnormal ultrasound examination. It revealed ren arcuatus, cardiac anomaly. Karyotype from amniocytes was 46,XX. QFPCR analysis confirmed normal STR pattern for chromosomes 13,21,X and Y using D13S258,D13S631,D21S11, D21S1414,X22,XHPRT STR markers. Female sex of the fetus was confirmed. D18S535 STR marker (18q12.2-q12.3) characteristics brought evidence of normal diallelic pattern with peak ratio 1,2. Repeated examinations of STR D18S51 marker disclosed diallelic type of partial trisomy 18 in 18q21.3 region with peak ratio 2,41 (2,31-2,60). The mother decided for the abortion of this fetus. In aborted fetus ventricular septal defect, open foramen ovale and ductus arteriosus, pericardial exudate, aplasia of umbilical artery, ren arcuatus with cystic dysplasia of the caudal kidney pole were found. These anomalies correspond to the described features of partial trisomy 18q21.1. The abnormal chromosome was inherited from the mother as follows from the comparison from parental and fetal electrophoretic patterns. These STR markers did not allow to ascertain origin in first or second meiosis, because the mother was homozygous for D18S51 STR marker. QFPCR provides exact, rapid diagnosis from microquantity of cells, determination of parental origin also in partial trisomies, non detectable by the current cytogenetic methods in the range of 270-310 bp as was in this case. *Supported by grants IGA 6462-3,6411-3,LN-00A079,11130003,00000064203*

P0994. Placenta/fetus discrepancies involving structural abnormalities of chromosome 8 detected in a prenatal diagnosis.

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Confined placental mosaicism is detected in 1-2% of pregnancies, but most of them involve aneuploidies. Structural mosaicism is a very rare event and difficult to interpret. We describe the case of a pregnant woman referred for prenatal diagnosis due to advanced maternal age. Semidirect cytogenetic analysis performed on chorionic villi showed a mosaic 46,XX,i(8q)/46,XX,del(8)(p11.2) karyotype, demonstrated by FISH. Karyotypes of the parents were normal. An amniocentesis was performed at 15 weeks, when ultrasonographical examination showed comunicant hydrocephaly. Cytogenetic analysis of cultured amniocytes showed a 46,XX,dup(8)(p23p11.2) karyotype. The pregnancy was terminated; pathologic findings included club feet, clenched left hand, subcutaneous edema and bilateral hydrocephaly. Molecular studies using chromosome 8 microsatellites performed on parents' blood and fetal tissues revealed a maternal meiotic origin of the inv dup(8p) with deletion of distal p23 region and duplication of remaining 8p, in agreement with other published cases (Floridia et al. 1996). We propose a model to explain the cytogenetic findings, which includes a first maternal meiotic error giving rise to a large dicentric isochromosome 8 present in the ovum, a second error in one of the first zygote divisions with misdivision of the dicentric 8 giving rise to a cell line with del(8p) confined to trophoblast and the othe cell line with inv dup(8) confined to fetal tissues, and a third error in trophoblast giving rise to a new cell line with isochromosome 8q.

P0995. Tetrasomy 12p or Syndrome de Pallister-Killian. Interest of the diagnostic on buccal smear

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We present a new case of 12p Tetrasomy diagnosed on buccal smear in the neonatal period.
A macrosomia was diagnosed during the gestation. A maternal diabetes was excluded.
At birth, the child was referred to NICU for hypotonia and neonatal distress.
Clinical evaluation showed a coarse and oedematous face, nuchal skinfolds, a mouth with downturned corners, dysplastic ears, a posterior cleft palate, an hypopigmented area of the scalp leading to the hypothesis of Pallister-Killian.
The neuroradiologic work-up showed a callosal dysgenesis with abnormal gyration pattern.
The diagnosis was prooved by direct FISH of buccal smear and controlled by fibroblast caryotype.
This case report illustrates the importance of perinatal US manifestations of PKS, the importance of caryotyping any foetus with unexplained macrosomia justified by the severe psychomotor retardation and the unfavourable pronostic.
Buccal smear allows easy and reliable diagnosis of this condition in a fraction of the time necessary for conventional caryotype on cultured fibroblasts.

P0996. Detection of trisomy 21 in a fetus during the investigation for Tay Sachs disease; prenatal cytogenetic study should be performed associated with molecular or enzymatic studies

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A family whose previous 2 children died from Tay Sachs disease applied for genetic counseling. Tay Sachs disease had been confirmed by enzymatic study in the second child and the mother was 6 weeks pregnant. They were told that prenatal diagnosis for Tay Sachs and cytogenetic study for chromosomal abnormalities in chorionic villus sampling (CVS) would be available at the 12th week of gestation. CVS was performed at the week of 12th gestation. Enzymatic study showed a normal fetus for Tay Sachs but cytogenetic study revealed a fetal karyotype of trisomy 21. Genetic counseling was given to the family and pregnancy was terminated according to the family's willing and ethic committee proposal at the 14th week of pregnancy.
Cytogenetic study should be offered in all pregnancies, which prenatal molecular or enzymatic studies are performed.

P0997. The informative analysis and prenatal diagnosis in the families with Duchenne muscular dystrophy in Moldova.

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Introduction: Duchenne muscular dystrophy (DMD) is a severe X-linked disease with an incidence of 1 in 3,500 males. Until recently, the most accurate diagnostic tests for DMD were the determination of serum creatine-kinase levels, muscle biopsy, and EMG. However, the application of recombinant DNA technology to the diagnosis of DMD has resulted in the development of more accurate tests.
Methods: 81 families with increased risk of DMD passed clinico-neurological, CK test, muscle ultrasonography investigations and molecular study. MPCR's were performed for deletion detection (18 different exons of dystrophin gene were tested in patients DNA) and RFLP-analysis (pERT87-8/TaqI, pERT87-15/BamH1 and 16 intron/TaqI polymorphisms).

Results and Discussion: About 76 % of probands were proved to be carriers of dystrophin gene deletion by MPCR. The given test highly is informative at the patients from Moldova. The analysis of informative families with MDD/B on the three intragenic polymorphisms pERT87-8/Tag1, pERT87-15/BamH1 and 16intron/Tag1 has revealed high of polymorphism 16intron/Tag1 (47,36 %) and low percent of informative was determined at the pERT 87-15/BamH1 polymorphism (21%). Smaller percent of informative has given polymorphism pERT87-8/Tag1 (41,77 %). These dates coincides with theoretical accounts of populations frequencies of the pERT87-8/Tag1, pERT87-15/BamH1 and 16 intron/Tag1 alleles which were counted by Hardy-Weinberg equilibrium.
Conclusion: The algorithm of molecular researches, selected by us, allows to define informative in 76 families (93% cases) and, accordingly, to conduct clinical, preclinical and prenatal diagnosis in DMD families.

P0998. Systematic follow up of 151 infants after diagnosis of nuchal anomalies at the 1st trimester

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AIMS: to assess developmental outcome of infants with nuchal anomalies at first trimester scanning and normal conventional karyotype.
DESIGN : Between 1994 and 2001, abnormal nuchal thickness was observed in 360 pregnancies. All measurement were performed by the same observer (EV). Nuchal anomalies were subdivided in nuchal thickening, nuchal translucency, and cystic hygroma. The fetuses were karyotyped, other anomalies were carefully sought and prenatal genetic counselling given accordingly.
RESULTS : For 184 fetuses, the parents finally elicited to terminate the pregnancy. 176 children were delivered (18 of them with scanning anomalies). All delivered babies were examined by one examiner (CB), and clinical follow up at 3, 6, 12 and 24 months was offered. 21 were lost to follow up. Among the remaining 151 newborns, 136 (90%) were considered to have normal psychomotor development at age 2. Among these, 21 (15%) had malformations (prenatally detected in 17 cases) : 12 isolated (8.8%) and 9 multiple (5.3%). In 15 cases, (10%) delayed psychomotor development was shown, either isolated (7 cases) or associated with identified (cyto)genetic syndromes (8 cases, 7 of them diagnosed postnatally). Ultrasonographic anomalies most predictive of an abnormal neurodevelopmental outcome were : nuchal hygroma, persistence of US anomalies in 2nd trimester, and presence of associated CHD.
CONCLUSIONS : Neonates presenting a nuchal anomaly during pregnancy are at high-risk for psychomotor developmental delay, even when neonatal evaluation appears “normal”.

P0999. Fraser syndrome : Report of 5 additional fetal cases and review of the literature

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The aim of this paper is a review of the diagnostic criteria of the Fraser multiple malformation syndrome (MIM 219000) in its severe fetal form. We based our study on the review of the literature, and report on the five additional fetal cases from two non-consanguineous sibships.
We reviewed 21 detailed fetal descriptions of Fraser syndrome from the literature (Thomas et al., Boyd et al., Ramsing et al.), and compared total of 26 fetal cases to 68 pediatric cases reviewed by Gattuso et al., the last representing viable milder form of the Fraser syndrome.
A quantitative estimate of the frequency of the principle clinical manifestations in the fetal versus pediatric population was obtained. The sensitivities of major criteria in fetuses varied between 77% (cryptophtalmos) and 96% (syndactyly), and of minor criteria varied between 81% (renal agenesis/laryngeal anomalies) and 23% (cleft lip/palate). Different renal anomalies were present in 96% of fetuses. Severe form of the syndrome should be more frequently diagnosed in-utero regarding a very poor prognosis and high perinatal lethality. Furthermore, a high recurrence risk of 25% among sibs warrants diagnosis for appropriate genetic counselling, the prenatal diagnosis being also much facilitated in further pregnancies in the cases with positive family history. Therefore, we are emphasizing the frequency of renal agenesis and laryngeal anomalies among fetal population and suggesting these two features should integrate major diagnostic criteria of Fraser syndrome in fetuses.

P 21. Sensory Genetics

P1000. Retinitis pigmentosa, metaphyseal chondrodysplasia, and brachyodactyly: an affected brother and sister

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A brother and sister, children of normal parents, are described. They had retinitis pigmentosa, causing near blindness as a result of very narrow fields of vision, associated with metaphyseal chondrodysplasia and marked shortening of the metacarpals and terminal phalanges.
Autosomal recessive inheritance is suggested with a common biochemical cause for all this defects.
This apparently new association of retinitis pigmentosa with a systemic bone dysplasia emphasis that this not common clinical diagnosis has a variety of different possible causes.

P1001. De novo translocation t (13; 18)(q14, q23) in a girl with disproportional short stature, congenital glaucoma, unusual face, persistent ductus arteriosus and muscular hypotonia

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Kamelia, K.S., a female patient, born at term(W-3000 gr.,L-46 cm.), from second uneventful pregnancy of unrelated young parents was investigated because of disproportional rhizomelic short stature and dysmorphic face, reminding hypo/ achondroplasia. The first pregnancy was aborted spontaneously. Otherwise, the family history was unremarkable.
At admission the patient manifested disproportional short stature with shortening of the long bones of limbs, high forehead with prominent tubera, depressed nasal bridge, low set dysplastic ears, hypertelorism, short neck, congenital bilateral glaucoma and congenital heart disease (persistent ductus arteriosus). Cytogenetic testing disclosed a de novo translocation t(13;18)(q14;q23) in the patient. Progressing shortening of long bones and initial metaphyseal changes of both ulnae (hypoplasia, irregularity, cupping of the ends) have been observed during 1 year follow-up.
Authors discuss the differential diagnosis and genetic basis of this unusual case of skeletal dysplasia, caused by structural chromosomal aberration.

P1002. Study Of The Wolfram Syndrome Gene (wfs1) In Spanish Patients With Diabetes Mellitus And Deafness

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Wolfram syndrome (WS) is an autosomal recessive neurodegenerative disorder characterised by early onset diabetes mellitus and progressive optic atrophy, as well as other clinical features such as deafness, diabetes insipida, renal tract abnormalities and diverse psychiatric illnesses.
A gene (WFS1) localized on 4p16.1 has been described, encoding a putative 890 amino acid transmembrane protein, WFS1. Recently,

a new locus for WS has been identified on 4q22-24, providing additional evidence for the genetic heterogeneity of this syndrome. This work aims to describe possible nucleotide changes in the WFS1 gene in a Spanish population affected with diabetes mellitus (DM, n=38), neurosensorial deafness (F, n=48) or both conditions (DM+F, n=48) vs. control group (C) of 48 healthy individuals.
We have identified a total of 18 nucleotide changes in the WFS1 gene: 3 mutations (D729N, L751I and V871M, the latter previously described), 4 new polymorphisms (1294C>G, 1308C>G, 1364C>T and 2438T>C) and 11 previously known polymorphisms. The analysis of the association of the polymorphisms with diabetes mellitus or deafness has revealed, when comparing DM and DM+F groups vs. control group, statistical differences in the allelic and genotypic distribution of the following changes: 1185T>C, 1832G>A, 2433G>A and 2565A>G.
Our data suggest that some changes in heterozygosis in the WFS1 gene can contribute to the diabetes mellitus and deafness phenotype, in the population studied.
This work has been supported by the MCYT, SAF 99-0079. E. Domènech has a FPI grant from MCYT.

P1003. Brachydactyly-Symphalangism-Deafness Syndrome (BSDS). A clinical-genetic study of 18 patients in two families

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Introduction: (Multiple Synostoses with Brachydactyly; Brachydactyly-Symphalangism Syndrome 186500, MIM) is a rare entity, and the diverse names reflect the uncertainty in the definition of the syndrome. Objective: The comparison of clinical-genetic aspects between the patients of two families and a correlation with data obtained from review. Patients and method: Our clinical-genetic study comprises 18 cases personally examined in two not connected families (a family with 14 cases, another family with 4 cases). Results: The cases offer a complete and well-defined clinical picture of a rare syndrome that has as definitory elements: brachydactyly+sy mphalangism+progressive deafness+autosomal dominant inheritance pattern. The syndrome is characterized by complete penetrance and variable expressivity. They are surprisingly similar in what the extension of finger morphologic lesions is concerned, but different in what the onset age and the degree of hearing loss is concerned. The large number of cases, on more generations, with constant association of the three anomalies, offers a good opportunity for gene investigation by linkage analysis. The role of Nog gene is discussed. Conclusions: The description of a large series of 18 examined cases belonging to two families shows the association of three distinct pathologic changes: Brachydactyly-Symphalangism-Deafness (BSD Syndrome). This definition of the syndrome, including all three pathologic changes, is distinguishing this syndrome from other resembling entities classified in the MIM catalogue.

P1004. Incidence of connexin-26 mutation 35delG and sensorineural hearing loss with unknown reason.

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Connexin-26 (Cx26) mutations account for 30-60% of non-syndromal hearing loss (NSHL) in European and American populations. Only one mutation 35delG is responsible for 70-80% of detectable Cx26 mutations. The hearing impairment has been described as severe or profound for these patients. Recently some studies confirmed a high carrier frequency, 2,14%, for 35delG mutation in Russia, but there was no data about frequency of this mutation between NSHL in the country.
We have studied 75 unrelated individuals with congenital NSHL in order to evaluate the prevalence 35delG mutation. Most of patients have had NSHL with unknown reason. We did not exclude patients with some risk factors for acquired hearing loss. In a total this mutation was found in 44% of the patients tested and was presented in 42% of sporadic cases.
The hearing loss in homozygous and heterozygous individuals varied from moderate to profound. No evidence of progression of the hearing impairment was found. The majority of homozygous for 35delG mutation have severe or profound hearing loss. Only one homozygous has significant asymmetrical hearing impairment. Two heterozygous patients have mild hearing impairment, but both of them are from deaf families.
We have confirmed that 35delG mutation of the Cx26 gene accounts for a large proportion of cases with congenital NSHL and especially in cases with unknown reason. More over hearing loss have considered to be acquired in some of 35delG homozygote before identification of this mutation. Thus molecular genetic analysis will allow appropriating genetic counseling. Other Cx26 mutations are under study.

P1005. Does molecular testing really improve genetic counselling ? example of Cx26 in non syndromic isolated deafness

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Hearing impairment is frequent : 2/1000 individuals present with deafness occuring in childhood. Half of which is now considered as having an hereditary origin. Around 70% of genetic cases are non-syndromic and most of them involve the connexin 26 gene. Here we present a multidisciplinary approach toward genetic counseling and molecular exploration in inherited deafness. Based on our experience, two types of situations are particularly critical and will thus be discussed.
One concerns the identification of a single mutation in CX26 among patients affected with profund, bilateral, prelingual deafness. In such a situation, genetic heterogeneity as well as a possible autosomal dominant mode of inheritance have to be considered. Besides, some mutations in CX26 have been reported as not being disease causing. Further explorations in non coding sequences (5' UTR exon, promoter, introns, enhancer…) have thus to be discussed. In addition, deletions in the Connexin 30 gene, adjacent to CX 26, have been reported in a digenic association in patients carrying heterozygous CX26 mutations.
The second point concerns the identification of disease causing mutations leading to a prenatal diagnosis request from parents. This aspect has to be carefully considered since the French National Ethic Comittee was unfavourable to termination of pregnancies in case of mutations in CX26. To date, in France, no pluridisciplinary center of prenatal diagnosis access to prenatal diagnosis in deafness. We wish to discuss the difficulties raised by the recent identification of genes in sensory disorders with regard to the improvement of genetic counseling.

P1006. Evaluation of Relation between Glaucoma and Antioxidative System (GST-M and GST-T) Polymorphisms

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Recent studies have been pointed out to the important role of oxidative mechanisms in the opthalmological diseases such as glaucome and cataract. Glutathione S-transferase (GST), one of the enzymes of antioxidative system, has an important function in defending against oxidative damages. μ (GST-M) and θ (GST-T) are the isoenzymes of GST whose polymorphisms are shown to affect the tendency toward different kinds of diseases. We searched the relation between glaucoma and GST-M and GST-T polymorphisms that play role in oxidative defense mechanisms. Results of 46 glaucoma patients (45-71 years of age) were compared with 53 normal control cases (48-68 years of age) lacking in an ophtalmological disease. Significant relation was obtained between GST-M null (with deletion) genotype and glaucoma (x^2 : 4.02 , p: 0.04) whereas there was no statistical significance between GST-T null genotype and glaucoma (x^2 :0.13 , p:0.71) According to this study assessing the pre-results of a wide-spread research, GST-M null genotype in Turkish population have an effect on tendency toward glaucoma.

P1007. Linkage analysis of a three generation pedigree with Autosomal Dominant Vitreoretinochoroidopathy (ADVIRC).

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Autosomal dominant vitreoretinochoroidopathy (ADVIRC) is a rare condition, which was first described by Kaufman et al. (1982). Lafaut et al reported a three-gene pedigree with ADVIRC, on which we have performed linkage analysis. A number of vitreoretinal disorders have been localised to a region of chromosome 11 (11p14-11q23) and include dominant familial exudative vitreoretinopathy 1 and 3 (EVR1 and EVR3), dominant inflammatory vitreoretinopathy (VMD2) and dominant neovascular inflammatory vitreoretinopathy (VRNI). Due to the relative richness of this region in retinal genes with overlapping phenotypes, initial analysis was performed for markers D11S1902 (11q13.3) and D11S527 (11q13.5). Further linkage analysis was performed yielding two recombinants. Evidence that the ADVIRC locus is centromeric to marker D11S929 was provided with a crossover in individual 5, in which segregation occurred with marker D11S922 (11p15.5) but not with D11S929 (11p14). A crossover in individual 10 at D11S4139 (11q13.3) indicates that the locus is centromeric to this marker. In this individual segregation occurred with D11S1362 (11q14.1) but not with D11S4139. Of the number of candidate genes in this region, the most likely to be associated with ADVIRC is PAX6. Normally associated with aniridia, PAX6 has been implicated broadly in human anterior segment malformations (Hanson et al 1994, Mirzayans et al 1995).

P1008. Nance-Horan syndrome: refinement of the gene localization on Xp22.13 and analysis of 5 candidate genes

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Nance-Horan syndrome (NHS) is an X-linked condition characterized by congenital cataracts, dental abnormalities, dysmorphic features, and mental retardation in 30% cases. Previous studies have mapped the disease gene to a 2 cM interval on Xp22.2 between DXS43 and DXS999. We report additional linkage data resulting from the analysis of 11 independent NHS families. A maximum Lod score of 9.94 (theta = 0.00) was obtained at the RS1 locus and a recombination with the locus DXS1195 on the telomeric side was observed in two families, thus refining the location of the gene to an interval of around 1 Mb on Xp22.13. Direct sequencing or SSCP analysis of the coding exons of 5 genes (SCML1, SCML2, STK9, RS1 and PPEF1), considered as candidate genes on the basis of their location in the critical interval, failed to detect any mutation in 12 unrelated NHS patients thus making it highly unlikely that these genes are implicated in NHS.

P1009. Linkage study and detection of a new mutation in PNR gene in large family with inherited retinitis pigmentosa

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Retinitis pigmentosa (RP) is a heterogeneous group of eye diseases, which includes some autosomal recessive and autosomal dominant inherited types of disorders caused by mutations in different genes. As usual the clinical history of RP starts within the first decade with night blindness, followed during the second and the third decade by slow reduction of the visual field. Here we are reported about large families from Voronezh province with inborn night blindness, hemeralopia, cataract and retinal pigmentary degeneration detected on first year of life. The parents are not consanguine and have 15 children, 6 of them are affected and one of these is newborn. We have carried out the linkage analysis with candidate genes and revealed the LOD score of 3.53 on chromosome 15 between D15S123 and D15S979 in 38 cM interval. The absence of recombination did not allow to refine this interval. The photoreceptor cell-specific nuclear receptor gene (PNR) is a candidate gene from this locus. The mutations in it are known in some cases of RP with late onset. We have screened PNR and detected the delA481 in maternal chromosome, which segregated with affected status. It is a first frame shift mutation in PNR; other reported mutations were in frame. We have not found the second mutation in this family in this gene during sequencing the coding part of paternal chromosome.

P1010. Linkage to 18qter differentiates two clinically overlapping syndromes: Congenital Cataracts Facial Dysmorphism Neuropathy and Marinesco-Sjögren syndromes.

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The Marinesco –Sjögren (MS) syndrome is an autosomal recessive disorder characterized by somatic and mental retardation, congenital cataracts and cerebellar ataxia. Peripheral neuropathy and acute rhabdomyolysis have been also occasionally described. CCFDN syndrome (Congenital Cataracts, Facial Dysmorphism and Neuropathy) is a recently reported condition considered to be a differential diagnosis of MSS (Tournev et al, 1999). CCFDN has been diagnosed, so far, only in a specific Gypsy group in Bulgaria. This disorder was mapped by linkage analysis to 18qter (Angelicheva et al, 1999).

We performed the clinical and molecular study of two consanguineous families with patients initially reported to have MS syndrome. The patients of the first family, originating from Turkey, had a classical MS syndrome especially with a pronounced cerebellar atrophy on MRI and an evocative muscle pathology. Linkage to 18qter was ruled out.

The three affected siblings of the second family, of Gypsy origin, differed clinically from the previous family by the presence of microcornea, marked peripheral neuropathy and a discrete cerebellar atrophy. The molecular findings were consistent with the diagnosis of CCFDN syndrome. Indeed, all three affected siblings were homozygous over four consecutive 18qter markers (D18S1122 to D18S70), confirming linkage to the CCFDN locus and excluding the diagnosis of MS syndrome.

This study emphasizes the clinical overlap between the MS and CCFDN syndromes which are however distinct genetic entities. Further molecular studies should lead to a better delineation of these syndromes.

P1011. ACE gene insertion-deletion polymorphism and risk of advanced retinopathy of prematurity in Kuwaiti Arabs

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Retinopathy of prematurity (ROP) is a disease characterized by neovascularization which occurs in infants with short gestational age and low birth weight and leads to retinal detachment and blindness. In a proportion of ROP cases, the disease progresses to advanced stages despite rigorous intervention. The genotypes for angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism were determined in 181 Kuwaiti premature infants by using a polymerase chain reaction (PCR) method. The incidence of I/D genotypes was compared in ROP cases (n = 74) and controls (n = 107) and within the two subgroups of ROP patients; one in which ROP regressed spontaneously (stage 1-3; n = 53) and two, in which it progressed to advanced stages (stage 4-5; n = 21). When the ROP cases were considered collectively, the incidence of DD genotype was almost identical to that in controls. The incidence of heterozygous ID genotype was higher in controls and the incidence

of II genotype was higher in ROP cases compared to controls ($p < 0.01$). In contrast to this, when ROP cases were divided in two subgroups the incidence of DD genotype was significantly higher in advanced stage ROP cases compared to spontaneously regressed ROP cases ($p < 0.04$). The incidences of ID and II genotypes were not significantly different amongst the two subgroups of ROP patients. The data suggests that the presence of DD genotype of ACE gene I/D polymorphism in Kuwaiti premature infants is associated with a higher risk of progression of ROP to advanced stages.

P1012. Linkage analysis in otosclerotic families

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Otosclerosis is the single most common cause of hearing impairment among white adults with a prevalence of 0.2-1%. Mean age of onset is in the third decade and 90% of affected persons are under 50 years of age at the time of diagnosis. Long-term follow up suggests that about 10% of these persons ultimately develop a profound neurosensorial hearing loss across all frequencies. The etiology of otosclerosis is unknown and its genetics is poorly understood. The majority of studies indicate autosomal dominant inheritance with reduced penetrance or heterogeneity or digenic recessive inheritance or digenic inheritance, but of an X-linked dominant gene and an autosomal recessive gene. Other studies suggest that otosclerosis may be a xenogenetic disease that requires a specific host genotype and exposure to a specific viral pathogen for the disease phenotype to be expressed.
To elucidate the pathogenesis of the otosclerosis, identification of the responsible genes is essential. Until now, genetic linkage in otosclerosis has been demonstrated only in two families. The first OTSC locus was identified on chromosomes 15q25-q26, and recently the second on chromosome 7q34-q36. Both OTSC loci are very large of 14.5 and 16 cM, respectively. We have selected Italian families with otoslerosis and we have realized a linkage exclusion/association analysis. Based on this strategy, we have excluded the linkage of some families to known OTSC loci suggesting the existence of at least one new OTSC locus. We have also evidence that one of our families is linked to OTSC1 locus.

P1013. Spectrum of connexin 26 gene (GJB2) mutations in families from Bashkortostan with inhereted non-syndromic hearing loss.

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Recent findings that a high proportion of non-syndromic hereditary sensorineural hearing loss is due to mutations in the gene for connexin 26 indicate the crucial role that the gene product plays for normal functioning of the cochlea. Mutations in the GJB2 gene account for the large proportion of pre-lingual hearing impairment with a prevalence up to 50% in autosomal recessive cases and still undefined prevalence in sporadic cases.
Ninety-seven subjects affected by non-syndromic sensorineural hearing impairment were unrolled in the study. The patients had either a family history of childhood hearing deficit or represented sporadic cases. Cx26 mutations we found in 58% of subjects. We identified the prevalence of the 35delG allele in the patients of sporadic cases with non-syndromic hereditary sensorineural hearing loss from Bashkortostan to be 52% of chromosomes screened. Screening the patients of 97 subjects we identified 66% 35delG mutation homozygotes and 20% heterozygotes. In addition to above mutations several types of mutations - del235G, 313-314delAA, 360delG, del314A were identified. The 35delG mutation was present in 86% of all Cx26 mutations identified. Also we observed two new mutations del314A and 360delG in the patients. The possible implication of the connexin genes mutations in the pathophysiology of some progressive adult deafness opens new prospects in the fine diagnostic of the ear diseases and eventually may lead to new therapeutic strategies applied to the cochlea.

P1014. Mutations in CLDN14 are a rare cause of non-syndromic recessive childhood deafness.

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Approximatively 50% of early-onset deafness cases are genetic and 50% of recessive cases are due to mutations in the GJB2 gene. The claudin CLDN14 gene on chromosome 21, encoding a member of the tight junction protein family, is mutated in two DFNB29 Pakistani families. To determine if CLDN14 mutations are an important contributor to the etiology of childhood deafness, we screened the CLDN14 single-coding exon on 366 chromosomes of non-syndromic recessive deaf patients from Spain and Greece, negative for GJB2 gene mutations. This study allowed us to define multiple polymorphisms in the CLDN14 gene.
An amino-acid substitution, G101R, found in a Greek patient in heterozygozity and leading to G101R is likely to be pathogenic. This variant has not been found in the remaining 365 chromosomes and affects a conserved residue of the claudin family. Gly101 is within the second transmembrane domain of this tight junction protein family and is preserved in 18 of the 20 described CLDN proteins. Unlike the modification observed in our patient the two remaining CLDN present small hydrophobic residues at this position (Ala and Val). R101 has been transmitted by the hearing father and cannot therefore be considered as a dominant mutation. We have investigated newly identified 5'UTR exons for a potential maternally-inherited mutation in this patient and found no potential second mutation. We are currently examining the pathogenicity of the G101R substitution in a functional assay. Our results indicate that mutations in CLDN14 do not substantially contribute to the non-syndromic deafness in the Mediterranean population.

P1015. Mitochondrial DNA mutations and deafness in the portuguese population

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Mitochondrial DNA mutations have been implicated in hearing loss, existing several mutations already reported in deaf families. However, despite the increasing number of reports implicating mtDNA mutations in hearing impairment, the frequency of these mutations as cause of nonsyndromic sensorineural hearing impairment (NSSHI) remains unknown. Some of those mutations, namely A1555G and A7445G, have been describe in different ethnic populations raising the possibility that such mutations are more frequent than initially thought. Screening for mtDNA mutations might thus be worthwhile in many cases of familial hearing impairment.
In the present study, we have analysed 30 NSSHI families with possible maternal inheritance, as well as 500 unrelated normal hearing individuals, for the presence of A1555G and A7445G mutations, by using restriction digestion and when relevant, direct sequencing of PCR products. Three other mutations affecting mitochondrial tRNASer (UCN) gene at nt 7472, 7510 and 7511 were also studied. The results obtained represent an additional contribution for the genetic characterization of the portuguese population.

P1016. Mutations in the connexin 26 gene in German, Hungarian and Polish patients with hearing impairment

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Mutations in the connexin 26 gene (GJB2) encoding a gap-junction protein have been identified in many patients with childhood hearing impairment (HI). One single mutation, 35delG, accounts for up to

70% of all analyzed European patients with HI inherited in autosomal recessive manner and 10% of patients with HI of unknown origin, respectively. Therefore we screened our collectives containing 291 German, 55 Polish and 90 Hungarian patients and corresponding control subjects for this and other connexin 26 mutations by PCR, SSCP and sequencing. For German patients with sporadic hearing impairment the 35delG frequency was 0.1 and for Polish cases 0.43, respectively. For Hungarian individuals the allele frequency was 0.41. All patients showing heterozygosity for 35delG or conspicous SSCP-results were sequenced. This study revealed several new patient-related mutations (e.g. G59V, D66A, I82M, K112E, R127H) and new gene variants resulting in amino acid substitutions. In summary, more than 20 new allelic changes were detected and for most of them, patterns of inheritance were documented.

P1017. Association of the T14709C mutation of mitochondrial DNA with maternally inherited diabetes mellitus and deafness in an Italian Family.

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A maternal effect in the transmission of non-insulin dependent diabetes mellitus (NIDDM) suggests the involvement of genetic factors encoded by mitochondrial DNA (mtDNA) in its pathogenesis. In this report, the np 3243, np 7445 and np 14709 mt mutations, and the 10.4 Kb deletion in 10 Italian families (Southern Italy) with maternally inherited diabetes mellitus and/or deafness have been analysed.The np 3243, np 7445 mutations and the 10.4 KB deletion were not found in any of the subjects of our study. Instead,in a family the mutation T14709C was found in three probands and in their diabetic mother. In this pedigree, a 16-year-old boy became deaf at the age of 12. The sister, who was diagnosed as having deafness at the age of 6, was affected by a severe myopathy and complained of progressive muscle weakness and exercise intolerance. A diagnosis of mitochondriopathy was based on a deltoid muscle biopsy that showed a large number of ragged-red fibres. The brother, a 10-year-old boy became deaf and developed diabetes at the age of 5 and 8 years, respectively.Their mother, a 60-year-old female was diagnosed as having NIDDM at the age of 57 years. The clinical phenotypes of our subjects carrying the T14709C mtDNA mutation are different. Identification of all mtDNA alterations or of interactions of mtDNA with nuclear genes would be required for the correct evaluation of the pathogenic mechanism in maternally inherited diabetes mellitus and deafness.

P1018. Prevalence of GJB2 and mtDNA mutations in childhood deafness in the Greek population.

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Mutations in the GJB2 gene have been shown as a major contributor to prelingual, sensorineural, non-syndromic deafness. Mitochondrial DNA (mtDNA) mutations have been reported in several unrelated families, but a more precise estimate of the prevalence of these mutations as causes of non-syndromic, childhood hearing impairment has not been well established. In the present study, patients were examined by an extensive questionnaire to exclude syndromic forms and environmental causes of deafness. The 35delG mutation was found in 42.2% of the chromosomes in 45 familial cases of prelingual, non-syndromic deafness (18 homozygotes and 2 heterozygotes) and in 30.6% of the chromosomes in 165 sporadic cases (45 homozygotes and 11 heterozygotes). Patients heterozygous for the 35delG mutation were analyzed by direct genomic sequencing of the coding region of the GJB2 gene, which revealed the W24X (2 alleles), L90P (2 alleles), 291insA (1 allele), and R184P (1 allele) mutations. The patients were also screened for the known mtDNA mutations A1555G, A7445G, 7472insC, and A3243G. The homoplasmic A1555G mutation was detected in two cases, one sporadic case and one family with hearing disabled members in both paternal and maternal lineages. The A7445G mutation was detected in one sporadic case of mild hearing loss. We conclude that GJB2 mutations are responsible for a large proportion of prelingual, non-syndromic deafness in the Greek population, and that routine screening for mtDNA mutations in congenital/childhood onset deafness is warranted.

P1019. Deletion of GJB6 in recessive non syndromic deafness

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Congenital profound deafness has a known genetic origin in more than 50% of the cases. If the majority of the non syndromic hearing impairment (NSHI) show an autosomal recessive inheritance associated to more than 25 loci, abnormal pattern in the GJB2 gene (connexin 26) is most commonly observed. Other connexin genes have been more rarely involved and attention was given to GJB6 gene (connexin 30). We have shown that a homozygous deletion of a minimal 250 kb region encompassing this gene causes NSHI. More strikingly, real time quantitative PCR has evidenced that the association of this deletion in trans of the GJB2 gene 35delG or E47X mutations is also associated with NSHI. Finding this deletion in several unrelated families prompt us to suggest that this mutation is not rare. To assess the origin of this deletion, haplotype analysis was performed in the DFNB1 region (encompassing the GJB2 and GJB6 genes): the preliminary data do not favor the hypothesis of an ancestral mutation. The cloning of the deletion borders is under progress. Sequence analysis of these regions should help to elucidate whether this deletion arise from a hotspot mutational event.

P1020. High frequency of 35delG mutation in Connexin 26 gene in Czech population and among Czech recipients of cochlear implantate.

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Mutations in Connexin 26 gene (Cx26) are the most common known genetic cause of sensorineural prelingual deafness. The mutation 35delG was reported to be by far the most common in Cx26. Most European patients with prelingual nonsyndromic deafness are homozygotes for 35delG.
No data about the heterozygotes frequency of this common mutation in Czech population were available befor our study.
In order to estimate the carrier frequency of 35delG in Cx26 gen in Czech population with normal hearing, we screened 503 randomly choosen DNA samples from healthy relatives of our neurologic patients and from patients with different neuromuscular disorders without hearing loss. Screening was done by allele specific PCR and positive samples were confirmed by fluorescent fragment analysis. 17 individuals out of 503 were heterozygous for 35delG, which represents a frequency of 1:29,6.
Further we tested 29 Czech patients - recipients of cochlear implantate, with nonsyndromic congenital - prelingual deafness with normaly hearing parents. 14 of them were homozygous for 35delG and 2 were heterozygotes, which represents that 51,7 % of investigated alleles were 35delG. Direct sequencing of all these 29 patients is in progress. First prenatal diagnosis for Cx26 mutation in Czech Republic was performed in a family with a congenitaly deaf child.
Our data are consistent with most European countries. To our knowledge these are first data about frequencies of Cx26 35delG mutation in patients with congenital deafness which received cochlear implantation. Supported by IGA and by VZ 11100003.

P1021. Connexin 26 Mutations In Neurosensory Non Syndromic Deafness

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Hereditary deafness occurs in about 1:2000 newborns and approximately 70% of the cases are non syndromic and the major proportion is due to autosomal recessive inheritance (80%) (Non Syndromic Recessive Deafness - NSRD). GJB2 is the gene more often involved and encodes the gap-junctions protein connexin 26 (Cx26). More than 60 different Cx26 mutations are described but one is particularly common, the 35delG, a deletion of a guanine within a stretch of six Gs residues that starts at position 30 of the coding region. The 35delG mutation accounts for about 60% of the mutated GJB2 alleles.
We analysed by direct sequencing the GJB2 the gene in 138 NSRD patients and identified mutations in 88/276 chromosomes analysed; 26.1% (72/276) showed 35delG mutation, while the remaining showed 12 different mutations (31del14, G12V, W77R, E47X, V95M, 310del14, 35insG, M34T, 167delT, L90P, D179N and H100L). We also found two allele variants: V27I and V153I (http://www.iro.es/cx26deaf.html). The 35delG was present in about 68% of all Cx26 mutations identified. Two out of twelve were novel mutations but while H100L was identified in a family with a recessive form of hearing loss, the D179N mutation was present in a subject where the subsequent family history showed a dominant hearing loss segregation.
In conclusion our findings confirm that the 35delG mutation of the GJB2 gene is the most common cause of NSRD in our population, but many other mutations are also present indicating that the complete sequence is needed for an appropriate molecular diagnosis and genetic counselling.

P1022. Mutations of TMPRSS3, the transmembrane serine protease causing deafness DFNB8/10 fail to activate the amiloride-sensitive epithelial sodium channel (ENaC) in vitro

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The TMPRSS3 gene which is mutated in autosomal recessive deafness DFNB8/10 encodes a transmembrane serine protease also containing LDLRA and SRCR domains. To investigate the expression pattern of TMPRSS3 we isolated the mouse ortholog. The mouse Tmprss3 gene maps to MMU17 and encodes a protein with 88% identity to the human ortholog. RT-PCR on rat and RNA in situ hybridisation on mouse cochlea, respectively, revealed that it is principally expressed in the spiral ganglion and epithelial cells supporting the organ of Corti and semi-quantitative RT-PCR on mouse tissues showed strong expression in the stomach. Transient expression of a TMPRSS3-ECFP protein in HeLa cells showed a primarily localization within the endoplasmic reticulum. The amiloride-sensitive sodium channel (ENaC) is regulated by channel activating serine proteases (CAPs). ENaC is expressed in many sodium reabsorbing tissues including the inner ear where it colocalizes with TMPRSS3 and is likely to be involved in maintenance of low sodium concentrations in the endolymph. Functional studies in a Xenopus oocytes expression system demonstrated that TMPRSS3 increased ENaC currents and that a significant fraction of TMPRSS3 is catalytically processed. In contrast, six TMPRSS3 missense mutations causing deafness that fall in all identified domains except the TM and a S401A mutant in the catalytic triad of TMPRSS3 all failed to activate ENaC and to undergo proteolytic cleavage. TMPRSS3-related deafness and these data indicate that important signalling pathways in the inner ear are controlled by proteolytic cleavage and that this may lead to abnormal sodium homeostasis of the inner ear.
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P1023. Expression of connexin 32 in the developing mouse peripheral auditory system

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Several connexin genes (*GJB2*, *GJB3, GJA1, GJB1* and *GJB6*) have been found mutated in patients with non-syndromic and/or syndromic deafness. *GJB1*, the gene encoding connexin 32 (Cx32) cause X-linked Charcot-Marie-Tooth syndrome, which is characterized by peripheral neuropathy, and in some cases progressive hearing loss. In order to understand better the physiology and the function of the connexins in the inner ear we have analyzed the expression of Connexin 32 (Cx32) by RNA in situ hybridization and by immunohistochemistry during the mouse cochlea organogenesis from early otocyst (E12) up to the mature organ in adult.
As previously described in the literature for other connexins, such as connexin 26, (*GJB2*), connexin 30 (*GJB6*), connexin 43 (*GJA1*) and connexin 31 (*GJB3*), various subtypes of fibrocytes, either in the spiral limbus or along the spiral ligament, differentially express Cx32. Also, we describe different levels of Cx32 expression within the sensory cells and within the various supporting cells that compose the organ of Corti. A huge expression is also present in the basilar membrane cells and in the Reissner membrane cells of ectodermal origin, as in subsets of the cellular elements of the cochlear ganglion. These data are discussed in relation to the cochlear development

P1024. Mutation screening of the CYP-1B1 gene in patients with primary congenital glaucoma, and among healthy Gypsies.

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Primary congenital glaucoma (PCG) is an autosomal recessive disorder associated with unknown developmental defect(s) in the anterior chamber, which leads to a severe eye disease. In the majority of PCG-cases, the disease causing mutation is located in the CYP-1B1 gene (GLCA3, cytochrom-P450-1B1). The type of the mutation shows large heterogenity in the Caucasian population. Recently a single ancestral mutation was found in an isolated Slovak Gypsy population of PCG-patients. All of the patients were homozigous for the Glu387Lys (G1505A) mutation in the CYP-1B1 gene, the carrier frequency was 10% among healthy Gypsies. The aim of our study was to identify the disease causing mutations in Hungarian PCG-patients. To predict the significance of the Glu387Lys mutation in Hungary, a population study was performed in two healthy Gypsy groups (Northern Hungary [NH]: n=207, Southern Hungary [SH]: n=150) by PCR-RFLP. We found significant differences between carrier frequencies of the two healthy Gypsy groups (NH: 2,9%, SH:0%). We analysed 24 PCG-samples (11 Gypsies, 13 non-Gypsies) for the presence of the Glu387Lys mutation and, found 10/11 homozygous Gypsy patients, while this mutation was not present among non-Gypsy patients. In the absence of the Glu387Lys mutation, direct sequencing of the coding regions are performed to identify the disease causing mutation. In conclusion, we found the Glu387Lys mutation in Hungary with significant geografical differences, which contradicts the Slovakian endemic theory, but confirms the Northern (Slovakian) Gypsy origin of the mutation. Among the Hungarian Gypsy PCG-patients, the most common disease causing gene defect is the Glu387Lys mutation.

P1025. Analysis of lmx1b gene in open angle glaucoma patients

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Nail patella syndrome is inherited in an autosomal dominant manner. The syndrome is characterized by dysplasia of the nails, hypoplasia of the patella, elbow dysplasia, progressive kidney disease and open

angle glaucoma.
Families have been studied whit OAG and NPS, but the results suggest that the NPS and OAG phenotypes in the families studied result from mutations in a single gene, lmx1b.
The gene lmx1b encoding the Lim homeodomain protein lmx1b plays a central role in dorso-ventral patterning of the vertebrate limb. The observation of a phenotype similar to NPS in mice prompted to consider lmx1b as a candidate gene for NPS.By the moment is not known the exactly mechanism of this gene.
We have studied samples of 92 patients by PCR and SSCP and identified three mutations in the coding region of the gene. />The mutations found are the following,in the exon 3 an insertion of a T in position 434 that leads to a stop codon. In exon 8, an heterozygous G--C transversion in position 1051 and in other sample, one insertion of G in position 997 leading to a stop codon.

P1026. Implications of the exons 1 and 2 of the MYOC gene in patients affected by open angle glaucoma

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PURPOSE: The myocilin/trabecular meshwork-inducible glucocorticoid response (MYOC/TIGR) gene was identified as a gene that cused open-angle glaucoma. Glaucoma is a group of disorders characterized by progressive excavation of the optic nerve head with associated loss of the visual field. Glaucoma is a higly prevalent disorder and is estimated to be the third most common cause of blindness worldwide. The mode of inheritance is autosomal dominant with reduced penetrance. The MYOC gene has been mapped at 1q23-q25. The MYOC gene consists of three exons; most known mutations map to the exon 3.
OBJECTIVES: Mutation identification in the exons 1 and 2. Implication of mutation in this patology.
METHODS: DNA from 92 patients with primary open-angle glaucoma was screened for PCR-SSCP analysis. Nucleotide sequence was determinated by automated sequencing.
RESULTS: Five mutations were identified in the exon 1, some of them have been mapped in the promoter region. In the exon 2 no mutations were present.

P 22. Techniques for mutation detection

P1027. Population screening for beta-thalassemia point mutations; development of a Micro-Array based Single Base Extension approach.

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Beta-thalassaemia is an autosomal recessive trait, which may occur at a high incidence in populations living in areas endemic for malaria. Carriership for this trait is believed to provide a selective advantage of survival after a *Plasmodium falciparum* infection, which is the inducer of malaria tropica. Homozygotes or compound heterozygotes, on the other hand, suffer from a severe microcytic hypochromic anemia known as beta-thalassaemia major, which results in early death (without proper treatment) or life-long transfusion and chelation therapy dependence. The majority of molecular defects causing beta-thalassemia are point mutations affecting beta-globin gene expression. The total number of different beta-thalassaemia causing mutations known to date is approximately 180. However a limited number of mutations occurs in different populations. Once the spectrum of beta-thalassemia-causing mutations is known for a certain population, a screening strategy for efficient diagnosis can be applied. The feasiblity of a micro-array based approach using Single Base Extension was tested in an immigrant-Dutch and an Iranian population from Hormozgan. A total of 18 different mutation-specific oligonucleotide primers were selected, covering approximately 90% of the Dutch and 86% of the Iranian beta-thalassemia mutations. The use of tagged-oligoprimers for mutation detection and subsequent hybridization to a generic micro-array containing covalently bound oligo-probes complemetary to the different tags, allows the simultaneous analysis of 27 frequently occurring single-base mutations in the beta-globin gene. We present the comparison of the results obtained by micro-array analysis and direct sequencing to discuss the feasibility of this approach.

P1028. Arrayed primer extension resequencing assay of TP53 tumour suppressor gene

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We have developed and evaluated APEX (arrayed primer extension) - based test for resequencing of TP53 tumour suppressor gene. Identification of TP53 gene mutations is important, because there is a correlation between the mutation type and the prognosis of cancer. Using the APEX test we can get full mutation data for the sequenced region of the gene at both DNA strands in a single assay.
A patient DNA sample is amplified, digested enzymatically, and annealed to arrayed primers, which promote sites for template-dependent DNA polymerase extension reaction using four fluorescently labelled dideoxy nucleotides. The TP53 gene test spans exons 2 to 9 plus introns 2 and 8. 98 individuals were analysed to obtain data on performance of the chip in a large-scale study. An average of 97,5% of the arrayed p53 gene sequence was identified from either sense or antisense strand and 81% from both strands. A common polymorphism in exon 4 (Arg72Pro) was found with minor allele frequency of 0.26.
Genomic DNA from 11 tumour samples was sequenced in a blind test. The results were predominantly concordant with TTGE (Temporal Temperature Gradient Electrophoresis) plus dideoxy sequencing. GenoramaTM imaging system and genotyping software were used for imaging and semiautomatic sequence analysis.
The fact that our assay can simultaneously perform mutation detection and correct identification of codon 72 status adds further weight to its usefulness as Arg72Pro polymorphism has recently been proposed to play a role in tumorigenesis.

P1029. Gene assembling, a new approach in mutation detection techniques - An application for BRCA genes scanning

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Many disease susceptibility genes are large and consist of many exons in which point mutations are scattered throughout. There has been an increasing demand for rapid and accurate method for scanning of point mutations in BRCA genes particularly.
Meta-PCR is a new method for creating chimeric DNA molecules using a modified PCR reaction (Wallace et al 1999) that allows maximizing the length of sequence that can be scanned by downstream technique.
Here we present data to demonstrate the assembles of exons 2, 20,23 and 24 of the BRCA1 gene and their subsequently analysis by direct sequencing.
The BRCA1 exons 2 and 20 are hot spot regions that are known to harbour particularly deleterious mutations. In order to avoid missing any mutation in these two exons, the above four exons were assembled in the following order of preferences: 23 20, 2 and 24. However, the order of fragments can be predetermined by primer design.
We verified by direct sequencing that the order and sequence of the component exons in the Meta-PCR products were as predicted. Meta-PCR products from three previously ascertained heterozygotes for BRCA1 mutations were directly sequenced and gave the same sequence patterns.
Scanning of each exon of BRCA1 and BRCA2 genes individually represent a tedious task. Meta-PCR technique might be circumvents this problem and is likely to be useful for clinical molecular diagnostic laboratories, helping them to fulfil the demand for scanning of complex genetic disease at the lower cost.
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P1030. Mutation Detection by High Throughput Single Strand Conformation Polymorphism using Automated Capillary Array Electrophoresis: Validation of Sensitivity.

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Detection of unknown mutations requires identification of any nucleotide substitution in a gene and is achieved through DNA sequencing. For large genes or large number of samples, this is a time-consuming process, necessitating methods that rapidly identify DNA fragments containing mutations, the exact nature of which may then be determined by DNA sequencing. One such screening method is Single Strand Conformation Polymorphism (SSCP) analysis, in which DNA fragments differing in only one nucleotide position may be separated on the basis of sequence-specific conformation of single stranded DNA. Polyacrylamide slab gel electrophoresis was originally employed for the single stranded DNA separation; recently, however, SSCP analysis carried out using capillary electrophoresis (CE-SSCP) has been introduced as a high-throughput screening method, and with the advent of multicapillary instruments, SSCP analysis using capillary array electrophoresis (CAE-SSCP) may increase the throughput several fold. In the present study, we have validated the mutation detection sensitivity of CAE-SSCP by constructing and analysing a panel of 68 mutants representing all types of substitutions as well as insertions and deletions in different sequence contexts in four exons from human genes. PCR amplicons, 150 to 300 bp in length, labelled with fluorescent dyes, were analysed by CAE-SSCP at three different temperatures (18, 25, and 35 °C) on a 16-channel ABI3100 Genetic Analyzer. Results were collected as data points and analysed with ABI Genescan and Genotyper software. The overall mutant detection level was found to be 96%, confirming the usefulness of this particular method for high throughput mutation detection.

P1031. Pyrosequence Analysis for Detection of Mutations Associated with Hereditary Hearing Loss in the Connexin 26 Gene and Mitochondrial DNA

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Hereditary hearing loss (HHL) is one of the most common congenital disorders and is highly heterogeneous. Mutations in Connexin 26 (CX26) gene account for about 20% of all cases of childhood deafness. This number approaches 50% in documented recessive cases of non-syndromic hearing loss. In addition, a single mitochondrial DNA mutation, A1555G, in the 12S rRNA gene, is associated with familial cases of progressive deafness. Effective screening of populations for HHL necessitates rapid assessment of several of these potential mutation sites. Pyrosequencing links a DNA synthesis protocol for determining sequence information to an enzyme cascade system which generates light whenever pyrophosphate is released during primer strand elongation. We assessed the ability of Pyrosequencing for detecting some of the common mutations causing HHL. Genomic DNA samples were collected from peripheral blood or dried blood spots using standard protocols. Mitochondrial DNA was coisolated with total genomic DNA. Detection of the most common CX26 mutations in individuals of Caucasian (35delG), Ashkenazi (167delT) and Asian (235delC, V37I) origins was confirmed by Pyrosequencing analysis. A total of 41 different mutations in CX26 gene and the mitochondrial A1555G mutation were also confirmed. Genotyping of up to 6 different adjacent mutations was achieved, including simultaneous detection of 35delG and 167delT. In addition, tests of the ability to quantitate Mt DNA heteroplasmy were successful. Major advantages of the Pyrosequencing approach include high throughput genotyping of disease-causing mutations, accurate and reproducible results, and assay flexibility. Experimental conditions can be optimized for a high degree of standardization and cost-effectiveness.

P1032. Diagnostic Mutation Scanning Using Microtitre Array Capillary Electrophoresis Fluorescent SSCP

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Mutation scanning can, in principle, provide a rapid screen of candidate exons or genes prior to a full sequence analysis, saving time and reducing costs. Single Stranded Conformational Polymorphism Analysis (SSCP) has been a popular method for scanning due to its simplicity (1). The only other method of comparable ease is Denaturing High Performance Liquid Chromatography (DHPLC)(2). We previously compared DHPLC with an improved Fluorescent SSCP (3). The two methods were of similar sensitivity. Here we report the adaptation of SSCP to a capillary array format the enables automated sampling and re-running of the same samples at different temperatures. Run times were reduced from 18 hours using the Applied Biosystems 377 to less than 1 hour using the 3100. We analysed 66 samples and controls from exons in hMSH2, hMLH1, p53, VHL and Menin using three different buffers (Tris-borate, Tris-taurine and Tris-MES) each at 5 temperatures. All mutations were detected, although to achieve this level of sensitivity we needed to use more than one buffer system. Some mutations were more "detectable" (that, seen under more conditions) than others. We used the MFOLD (http://bioinfo.math.rpi.edu/~zukerm/) single stranded DNA structure analysis program to investigate the detectability of various mutations with the aim of making SSCP more predictable.

1. Orita M et al (1989) Genomics 5 (4):874-879
2. Underhill PA et al (1997)Genome Research 7:996-1005
3. Ellis LA, Taylor CF, and Taylor GR (2000) Human Mutation 15:556-564.

P1033. Mutation detection assay for ABCR (ABCA4) Gene, based on Arrayed Primer Extension technology

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The ABCR gene arrayed primer extension (APEX) assay was designed for the detection of over 300 variants currently described in ABCR (ABCA4) gene. Mutations in the gene are associated with at least five inherited retinal dystrophies: Stargardt disease (STGD), fundus flavimaculatus (FFM), cone-rod dystrophy (CRD), retinitis pigmentosa (RP) and age-related macular degeneration (AMD).
We have used Arrayed Primer Extension (APEX) technology for identification of ABCR gene mutations as an alternative to mutation detection based on SSCP plus dideoxy sequencing. The genomic DNA sample is amplified and annealed to arrayed primers, which promote sites for DNA polymerase extension reactions using four fluorescently labelled dideoxynucleotides. The oligonucleotide array scans a total of 349 mutation sites (the vast majority of mutations currently known in the ABCR gene) from both strands. The ABCR chip was validated with 150 STGD patients and 100 controls in a blind test. The same DNA samples were previously analysed with the SSCP technology. Differences in SSCP and chip results were controlled by sequencing. The APEX based technology determines the existing genetic variation with 97% efficiency.
The APEX method provides efficient tool for mutation and polymorphism analysis both for scientific and clinical research.
The ABCR gene APEX assay can be applied as screening tool in ophthalmic genetics.

P1034. Rapid detection of DMD/BMD carriers by quantitative Real-time PCR

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Two thirds of Duchenne and Becker (DMD/BMD) muscular dystrophy cases are due to deletions in the dystrophin gene on Xp21.
While most deletions are readily detected in patients by PCR, the identification of female carriers in affected families remains difficult in some cases, e.g. when no informative markers are available in the deleted region.

Using the Light cycler technology we have developed a simple and rapid assay for the direct detection of carriers of exon 44, 45 and 49 deletions in the dystrophin gene. Primer pairs for each exon were designed to give specific and efficient PCR under identical conditions. The products were quantified using SybrGreen and the ratios between the potentially deleted exon and a non-deleted reference exon were calculated. We have analysed 16 known carriers and 16 non-carriers in 8 independent assays: in all cases the correct status was reliably detected (mean ratios: carriers 0.52, non carriers 1.11). There was no overlap between the ratios of carriers and non-carriers: carriers ranged from 0.33-0.69, non-carriers from 0.85-1.35.
We are currently adapting the assay to a number of other exons frequently deleted in the dystrophin gene and we are testing the possibility of adapting the protocol to other conditions involving gene deletions or duplications such as CMT/HNPP and NF. The method has the advantage that no specific labelled hybridisation probes are needed. It offers a reliable, rapid and inexpensive possibility for the detection of heterozygous carriers of deletions in the dystrophin as well as in a number of other genes.

P1035. A rapid, accurate and quantitative method for analysis of the methylation status of imprinted genes, using primer extension and IP RP HPLC.

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We have developed a rapid, accurate and quantitative method for the detection of methylation differences at specific CpG sites based on bisulphite treatment of DNA followed by primer extension and ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC). The application of the method is illustrated by analysis of differentially imprinted alleles arising from Prader-Willi and Angelman syndromes.
In order to convert unmethylated cytosines to uracil, plasmid and genomic DNA samples were treated with sodium bisulphite and the targeted sequence was then amplified using oligodeoxynucleotide primers specific for the bisulphite-deaminated DNA. The PCR product(s) from this step was used as a template for a primer extension reaction and the products were subsequently analysed chromatographically using IP RP HPLC. This method eliminates the need to use restriction enzymes to determine the methylation status of the amplicon and also circumvents the need for radio labelling for the quantitative measurements. Finally, this method removes the need for nucleotide sequencing since it is not solely reliant on the presence or absence of one or more PCR products, as is the case with related methods.

P1036. Fluorescent analysis of microsatellite markers using a unique fluorescent primer : a simple technique validated on 55 CA or tetra repeats

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Study of microsatellite (CA or tetra repeats) segregation around a locus is a tool widely used. Traditionally, such a study is performed with two flanking specific primers, one being labelled (radioactive or fluorescent) for detection. Use of radioactivity is inconvenient in a diagnostic setting, and implies an additional step of kination for each microsatellite analysed. For fluorescent analysis on automated sequencers, labelled primers are commercially available, but are rather costly and have a limited lifetime. This aspect is a real limitation when such primers are used infrequently for genetic counselling of very rare diseases.
For each microsatellite, we use its two unlabeled specific primers, and a unique fluorescent primer, in an single reaction. One of the specific flanking primers is tailed with a common 5' sequence selected by Warner et al (1996) for the TP-PCR technique. Primer sequences were chosen in order to define the best combination between one of the specific primer and the other with the common tail. Detection is achieved with a fluoresceinated primer corresponding to this common sequence. In the early amplification cycles, specific primers give rise to a product with the tail. A particular molar ratio of primers ensures that the specific primer with the tail is exhausted in the early amplification cycles. This allows the fluoresceinated primer to amplify preferentially from the end of products from previous amplification rounds.
We have validated this strategy for 55 microsatellites, most of them on the X chromosome. We have tested successfully multiplex for up to 4 microsatellites.

P1037. Pure sample preparation: A prerequisite for high quality molecular analyses

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State-of-the-art molecular analyses need the handling with extremely pure samples to yield a good result. This is important e.g. for single cell genetic analyses (like in fetal cells in maternal blood, in preimplantation diganosis or in disseminated tumor cells), for microarray technologies and for single chromosome preparation. Thus pure sample preparations are indispensible for various fields in medicine and biology.
To obtain pure samples is one of the most thrilling tasks in modern molecular science. An up-to-date tool in this topic is Laser Microdissection combined with Laser Pressure Catapulting. This laser technology simply utilizes the force of focused laser light to eject a selected specimen from the object plane and to directly lift it into the cap of a routine microfuge tube. This completely non-contact Laser Pressure Catapulting technology avoids any danger of contamination with unwanted specimen. In every case where the comparison of different cell types (genetic, expressional or proteomic) is important for research or diagnosis, a precise differentiation between selected cells is mandatory.
Any kind of tissue from various sources (also archival histological samples or living cells) and even subcellular structures can be captured using this laser method. Wherever precise micromanipulation is required or where the procurement of homogenous samples is obligatory for the subsequent analysis of specific genetic or proteomic alterations, the PALM MicroLaser system is a key technology.

P1038. The GenoSNIP assay - A novel method for SNP genotyping by MALDI-TOF mass spectrometry

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After completion of the Human Genome Project, intensive exploration of diversity in different individuals is one of the main objectives in human molecular genetics. In particular, analysis of single nucleotide polymorphisms (SNPs) is expected to have an enormous impact in future human medicine, e.g. by determination of risk factors for common diseases, causes of multifactorial diseases, and modulators of pharmacologic effects.
MALDI-TOF mass spectrometry has been shown to be one of the most valid methods for SNP genotyping. Combination of analysis speed, accuracy of results, cost-effectiveness, and automation capabilities makes MALDI-TOF one of the most promising SNP-typing technologies for medical research and diagnostics. On the other hand, drawbacks of the technique are complex sample preparation and high purification requirements before MALDI measurement.
Here, we present a novel method for MALDI SNP typing sample preparation, GenoSNIP. An UV-cleavable site is introduced into a primer which hybridizes adjacent to the polymorphic site. This photolinker creates an abasic site in the primer and does not prevent annealing to the target sequence. The primer is converted by single nucleotide extension to products specific for the corresponding alleles. Subsequently, these products are cleaved by UV light resulting in very small molecules, usually pentamers, which can be measured with very high sensitivity, accuracy, and resolution in a MALDI-TOF MS instrument. We present different designs of the GenoSNIP assay including a simple pipetting protocol which is fully compatible with the 384 microtiterplate format. The genotyping of several common genetic risk factors is demonstrated.

P1039. A Comparison of Software Tools for Comparative Sequencing

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The release of version 1.1 of SeqScape™ software marked the introduction of basecalling quality values and assembly-based SNP identification into a desktop comparative sequencing software tool. SeqScape™ software is compatible with Windows NT®/Windows® 2000 OS and contains fully integrated basecalling, trimming, sequence assembly, alignment, and sequence comparison tools for fast and accurate sequence comparisons and variant identification. We present here a comparison of the features, ease of use, and robustness of algorithms between SeqScape™ software and other available comparative sequencing software tools. We also critically evaluate the accuracy of analysis results from SeqScape™ software version 1.1 versus Sequencher™ software. Highlights are presented of new algorithm features in development for the next version of SeqScape™software, including the ability to detect and identify heterozygous frameshift mutations using direct sequencing.

P1040. Multiplex PCR of Short Fluorescent Fragments: a simple, fast and reliable method for the detection of heterozygous genomic rearrangements

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The detection of heterozygous genomic deletions and duplications is technically difficult and represents a serious limitation to the complete diagnosis of many genetic diseases. We have developed Multiplex PCR of Short Fluorescent Fragments, a simple method which is based on: (i) the simultaneous amplification of several short genomic sequences using fluorescently labeled primers, (ii) the use of a limited number of cycles, (iii) the superposition of the fluorescent electropherograms and, (iv) comparison, between patients and controls, of the peaks representing the fluorescence of each amplicon. This method has already been adapted to the following genes: *MSH2*, *MLH1*, *MSH6*, *C1NH*, *SMN*, *BRCA1* et *RB1*. It has already been included into our diagnostic routine of the HNPCC syndrome, of the hereditary forms of breast and ovarian cancer and of retinoblastoma and has improved the genetic counseling of spinal muscular atrophy. Moreover, this method has allowed us to characterize precisely the boundaries of a large number of heterozygous deletions or duplications. This method is much more sensitive and rapid than the Southern blot technique commonly used, is better suited than quantitative real time PCR to the analysis of genes containing large numbers of exons, and appears to be more flexible than the *MAPH (Multiplex Amplifiable Probe Hybridization)* method, particularly because it can be rapidly adapted to large numbers of genes.

P1041. DNA sequence analysis using ten-mer oligonucleotides microarray and allele-specific primer extension.

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The aim of this project was to test 10-mer oligonucleotides for sequence analysis by arrayed primer extension (APEX) method in microarray format on glass slides.
Firstly, 256 oligonucleotides, consisting of constant GAATTC part in 5'end and a variable 3' end part, containing all possible tetramers, were chosen. Twenty synthetic oligonucleotides of 50bp length, containing EcoRI restriction site, were used as templates in this system. Reaction mixture for APEX contained all four fluorescently labeled ddNTP-s, thermostable DNA polymerase and denatured templates. During optimization different template concentrations were tested at temperatures ranging 20°C - 45°C. The nucleotide in the fifth position from the EcoRI site was correctly identified in all templates.
Secondly, to further improve APEX specificity with thermostable DNA polymerase, we tried to raise Tm's of 10-mers by including one, two or three LNA monomers into different positions of three oligonucleotides. The reactivity of original and modified oligonucleotides was tested in APEX and hybridization experiments at temperatures ranging 30°C - 50°C. Fluorescence signals were scanned by Array Scanner 428 and quantitated by ArrayPro Analyzer 4.0 software. Contrary to expectations, LNA oligonucleotides produced weaker signals than normal 10-mers in APEX reactions. At the same time LNA oligonucleotides gave equal or 2-6 times higher signals in hybridization. Probably LNA monomers interfere in DNA polymerase reaction with such oligonucleotides.
In conclusion, 10-mers could be effectively used for APEX method in oligonucleotide microarray format on glass slides. Addition of LNA monomers into DNA oligonucleotides did not improve performance of these oligonucleotides in APEX reactions.

P1042. Applications Of Genetic Analyzer (genescan) In The Diagnosis Of Genetic Diseases

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Diagnostic methods for genetic diseases have suffered great development in the last years because of the technologic advances. One of these new methods is based in an automated genetic analysis by capillary electrophoresis.
The ABI Prims® 310 Genetic Analyzer (Applied Biosystems) make use of two software packages: Sequence Analysis and GeneScan. The GeneScan program is used in our laboratory to analyse the fragments obtained after DNA amplification by a PCR using fluorescence-labelled primers:
Indirect studies: haplotypes analysis by small tandem repeat (STR) markers in 56 families.
- Duchenne/Becker Muscular Dystrophy.
- Prader Willi and Angelman syndromes.
- Charcot-Marie-Tooth 1A /Pressure Palsie (HNPP).
- XLRP.

Rapid detection of the major chromosome aneuploidies (13, 18, 21, X and Y) and fetal sex determination in 47 cases.
Direct studies: expansion of unstable repeats in 34 cases.
- Huntington´s disease.
- Myotonic dystrophy.
- Fragile X syndrome.

Similar results were obtained when compared with previous conventional methods.
Therefore GeneScan program allows a reliable and reproducible analysis.
Besides, other advantages of this technique are:
- More accuracy distinguishing homozygote from heterozygote status.
- More authomatic and easier technique.
- Avoiding the use of radioactive products.

This technology has led to the innovation and improvement of the diagnostic methods.

P1043. SeqScape™ Software: Further advances in Applied Biosystems' Sequence Comparative Analysis Tool for Variant Identification

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In biological research, the end goal is obtaining a high quality result in a timely and cost effective manner. Applied Biosystems has developed more efficient and handsfree methods by which this sequence data can be obtained with the development and improvements to automated sequence analysis. Following in this vein, downstream applications are required to allow the researcher to fully analyze and understand the data generated. Further advances in data analysis with SeqScape™ software v1.1 now mean this goal is accomplished in an organized and competent way. The next revision of the software tool is in progress which includes improved functionality of the Reference Data Group, the use of discontinuous numbering, better definition of Intron/Exons and Primer locations as well as features to support haplotyping and ORF recognition. These, coupled with the implementation of library searching and tree drawing tools will benefit a larger range of comparative analysis researchers. Additionally, access control and an audit trail are becoming more important in the realm of pharmacogenomics. By providing these sophisticated capabilities the user achieves a more valuable and comprehensive analysis of their data and progresses faster to an effective outcome.

P1044. Predicting the Optimal PCR primer annealing temperature for use with the Transgenomic Optimase™ polymerase.

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Optimase™ polymerase is a novel proof reading polymerase. The high fidelity of this enzyme finds particular applicability to mutation detection using Denaturing High Performance Liquid Chromatography (DHPLC). Empirical determination of the optimal primer annealing temperature (Ta) during PCR has been carried out using 29 different human genomic primer sets for Optimase polymerase (Transgenomic Inc), Ampli Taq Gold (Perkin Elmer) and Pfu (Stratagene) using optimal conditions for each enzyme and a gradient thermal cycler (Hybaid). The results from this work enable a simplified strategy for successful prediction of the primer annealing temperature to be determined. In summary prediction of the primer Tm using an equation that takes into account the monovalent salt concentration, primer length and GC composition is critical and is different for each enzyme buffer composition. In addition we indicate the necessity to distinguish between primer Tm and Ta. We show here for the Optimase™ DNA polymerase an ideal where the Ta is 2-3°C higher than the predicted Tm for optimal PCR amplification.

P1045. Demonstration of Hot start characteristics in Transgenomic Optimase™ Polymerase

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The use of so-called "Hot Start" techniques has been employed to improve specificity, sensitivity and yield of the polymerase chain reaction (PCR). Although efficient amplification by PCR enzymes is optimal at elevated temperatures there is a possibility that some amplification may occur during sample set up particularly if this procedure is carried out at room temperature. For this reason the choice of a "HotStart " enzyme is thought to convey a significant advantage. Numerous permutations of this technology are available including omitting an essential component until the tubes are at 70oC, physically separating the components using a barrier material such as a wax plug and enzyme inhibition by the use of antibodies or chemical inactivation. We have isolated, characterised and developed a novel proof reading polymerase (Optimase™ polymerase). This high fidelity DNA polymerase has been shown to have minimal activity at ambient temperature. We demonstrate the reality of routinely setting up PCR reactions at room temperature and also being able to store samples for twenty four hours, prior to cycling, with no adverse effects on yield, misincorporation rate or amplification specificity.

P1046. Multiplex capillary heteroduplex analysis (MCHA): A rapid and sensitive method for detection of mutations in Bardet-Biedl syndrome genes.

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Background
Six Bardet-Biedl syndrome loci (*BBS1-6*) have been mapped and three identified (*BBS2, 4* & *6*). Inheritance in some families is complex with multiallelic participation, making linkage unreliable. These factors hamper our ability to efficiently screen for mutations in BBS patients.
Aims
To develop a rapid and cost-effective mutation assay for *BBS* genes.
Methods
PCR amplification using fluorescent oligonucleotides for *BBS2, 4* & *6* are denatured with like wild-type PCR products to generate potential heteroduplexes to be analysed on the MegaBACE 1000. Throughput can be greatly enhanced by multiplexing compatible fragments. Between 70 and 95 Bardet-Biedl pedigrees were analysed for the presence of heteroduplex formation.
Results
We adopted two approaches using MCHA; 1. "blind" screening of BBS pedigrees for mutations in *BBS4* and *BBS6* followed by sequencing. 2. screening for known mutations in pedigrees previously sequenced for *BBS2* mutations.

1. We detected 25 changes in *BBS4* and 18 changes in *BBS6* subsequently revealed to be SNPs or novel mutations.
2. Screening of *BBS2* revealed 6 changes including a two-base deletion and single base substitution, a 100% detection-rate.

Conclusions
MCHA is superior to slab gel HA in particular its ability to detect point mutations. The assay is simple and rapid with up to 96 samples analysed in 40 minutes permitting resolution of fragments up to 650bp thus reducing the overall number of analyses required. We have found adaptation of HA provides a rapid and accurate screening method for mutation detection in BBS.

P1047. Comparative sequence data analysis using SeqScape™ software for high throughput mutation scanning in DNA diagnostic analyses

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The identification of disease causing small mutations in human genetic diseases is generally performed using indirect mutation scanning techniques such as SSCA, DGGE or DHPLC and subsequent sequencing of PCR amplified products. Direct sequencing is relatively straightforward and therefore suitable for automation of the complete procedure from reaction setup to purification of PCR products and sequencing reaction. The availability of automated pipetting stations and large capacity DNA sequencers enables the use of this method for high throughput mutation scanning. However, a major drawback of this approach is the large amount of sequence data to be analyzed both efficiently and accurately. Furthermore, the available sequence analysis software is usually not developed for comparative mutational analysis and therefore not entirely suitable. Recently the SeqScape™ software (Applied Biosystems) was introduced, allowing base calling, sequence assembly, alignment and comparison, all combined in one analysis. Prior to the analysis, for each amplicon a fragment specific analysis definition is setup containing analysis defaults, reference sequence and base variants previously identified. This results in clean sequence data and easy identification and characterization of homo- and heterozygous base variants and has led to a significant reduction in manual data editing in our laboratory. However, for correct identification of heterozygous deletion or insertion mutations, extended data management and quality assurance improvements are required. This approach has enabled us to standardize our mutation detection method and perform more than 2000 DNA diagnostic tests annually in over 700 amplicons and 50 different genes in an efficient and accurate procedure.

P1048. Automated screening by DHPLC detects LDLR mutations in FH patients from New Zealand

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Familial hypercholesterolaemia (FH) is a common inherited disease, with premature vascular disease occurring 10-20 years earlier than in polygenic hyperlipidaemia. FH is easily and effectively treated. Although founder effects occur, countries with heterogeneous populations have an array of mutations; thus a gene screening approach is the appropriate first step for mutation detection. Methods previously used to screen for LDLR mutations lack sensitivity, therefore samples from patients in NZ are being screened for LDLR mutations by denaturing HPLC (DHPLC). DHPLC has numerous advantages: analysis is rapid, inexpensive and automated. Sensitivity and specificity range from 96-100%.
Seven patient samples plus controls are screened simultaneously in a largely automated process. After PCR setup in two microtitre plates by a Tecan robot, all exons are amplified under one set of conditions. Samples are then analysed by DHPLC and duplicate abnormal products sequenced by direct sequencing.
Eight novel mutations were characterised from 27 different mutations (in 52 patients). Seven novel mutations are in the EGF domain, with the eighth in the membrane-spanning domain. The majority of

mutations are localised in the EGF domain and not the ligand-binding domain as previously reported.
The diversity of LDLR mutations highlights the importance of analyses that target both known and novel mutations, especially in heterogeneous populations. As effective clinical management of FH is aided by early diagnosis, mutation detection programmes must take into account the incidence of novel mutations. Automated setup for DHPLC appears ideally suited to LDLR mutation analysis in clinical and research settings.

P1049. Novel Sample Preparation Technology for Rapid, High-Throughput Purification of Genomic DNA from Blood and Tissue Culture Cells

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With the explosive growth in disciplines such as genomics, diagnostics, transfusion medicine and human ID there is an increasing need for fast, simple and high-quality sample preparation systems. New developments in filter media are bringing about a revolution in DNA isolation, purification and storage procedures. Using our expertise in filtration technologies, Whatman has developed a range of novel and complementary systems for the purification of nucleic acids from whole blood and other sample types. These unidirectional vacuum-based systems offer maximum flexibility, enabling purification of DNA from up to 10 ml of blood in 30 minutes whilst minimising risks inherent in multiple-step extraction protocols. A new 96-well format is now available (Gen96) which is designed to process small volume blood samples (5-75 µl) and tissue culture cells (2500 – $1x10^6$ cells per well) in under 30 minutes. Genomic DNA recoveries of up to 90% of total are observed and the gDNA yielded is of high quality, suitable for PCR based techniques including STR analysis. Gen96 incorporates patented FTA™ technology, lysing cells on contact. Once the sample has dried this technology protects the immobilised gDNA from degradation, bacterial colonisation, UV and free radical damage. Viruses and other microorganisms are inactivated, offering user safety and simple shipment of samples. The system is therefore ideal for remote sampling applications, with samples able to be stored at room temperature for months prior to extraction without loss of DNA integrity or performance.

P1050. High sensivity and specificity of denaturing high performance liquid chromatography (DHPLC) for mutation analysis of the FBN1 gene in patients with Marfan syndrome.

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Marfan syndrome is an autosomal dominant inherited disorder of the connective tissue that principally involves the cardiovascular,ocular and skeletal systems. The incidence is estimated to be 1:5000, with 25% sporadic cases. The leading cause of death is related to the cardiovascular involvement, in particular aortic root dilatation and rupture.
The disease is caused by alteration in FBN1 gene (65 exons, located at 15q15-q21.1). Causal mutations are scattered throughout the gene and are largely unique to individual families.
The FBN1 gene was analyzed in 29 unrelated patients suspected to be affected by Marfan syndrome. To develop an efficient and faster method capable of identify all possible mutations in this gene, we introduced DHPLC technology in the analysis of 25 exons in which mutations recur. We first analysed the FBN1 exons and exon-flanking non coding regions gene coding regions with automated sequencing of all 65 exons (ABI PE- 373 DNA Sequencer) to identify mutations and polymorphisms. Then, DHPLC analysis was carried out on the WaveTM DNA Fragment Analysis System (Transgenomic, Cheshire, UK). DNA fragment elution profiles were displayed using the Transgenomic WAVEMAKER-TM software. Chromatograms were analysed and amplified fragments showing alterations were re-confirmed by automated sequencing. Overall, by direct sequencing we indentify 19 variants (14 in coding regions and 5 in intronic sequences). A corresponding number of heteroduplex profiles was detected with DHPLC with 100% correspondence to the variant-containing regions previously identified by direct sequencing. Our results confirms that DHPLC is a highly sensitive and specific technology for DNA sequence variant detection.

P1051. APEX scaling up. Optimisation of the high throughput Arrayed Primer Extension based DNA variation analysis for accurate and affordable large-scale projects.

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The hybridisation of complementary strands of DNA is the underlying principle of all microarray-based techniques for the analysis of DNA variation. Arrayed Primer Extension based microarray technology puts Sanger sequencing, where a polymerase is used to extend one strand of DNA primed by other strands, on a chip. The oligonucleotides arrayed on a glass chip hybridise to the sample DNA, then a polymerase and fluorescent terminator nucleotides are added. Four colours are used, each corresponding to a DNA nucleotide. The arrayed oligonucleotides are synthesized so that they are just one nucleotide from the site of the expected mutation. Incorporation of the dye terminator nucleotide gives us an exact readout of what is at the site of the mutation. This dual selection feature gives the chip a higher signal-to-noise ratio for increased reading accuracy compared to the traditional hybridisation only chip. Asper Biotech has been running routine custom assays with 3,000 different oligos on a chip, but we are nowhere near the limit. Along side with the substantial increase of the number of oligos of the chip, application of multiplex PCR of ultimate importance in reduction of the assay cost-per-SNP. We have devised proprietary software for prediction of matching and grouping the hundreds to thousands of primer pairs before the start of the wet lab experiments.
In the current poster presentation we demonstrate the performance of our in silico results through PCR lab to the chip, as well as piling up smaller subarrays to a large chip with increased complexity.

P1052. Deletion/Duplication Detection In The 79-Exon DMD-Gene Using A Porous Micro-array System.

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DNA deletions and/or duplications are a frequent cause of genetic disease. Detecting these is a critical aspect of DNA diagnosis, yet due to technical problems this analysis is often not performed. We have tested Multiplex Amplifiable Probe Hybridization (MAPH) as a tool for detecting such mutations in the DMD-gene, which cause Duchenne and Becker Muscular Dystrophy (DMD/BMD). MAPH is a quantitative technique, with probes being recovered by PCR following hybridization to immobilized genomic DNA.
We have analyzed the PCR-products using micro-array technology, in particular porous microarrays (PamChip™). Compared to planar arrays, the PamChip™ has several advantages, including the larger active surface area, the possibility to vary hybridisation stringency during analysis and significantly decreased hybridization times.
60-mer oligos specific for individual exons were spotted on the PamChip™ substrate. Following MAPH the PCR products were fluorescently labeled and hybridized on the microarray. Sufficient signal was obtained within 10 minutes. By comparing spot intensities between controls and patients it was possible to detect deletions and duplications in both males and females, including one and two exon duplications that were missed by other techniques. Preliminary results show a high level of reproducibility, suggesting that even smaller changes might be detectable, including mosaic cases.
This proof-of principle has encouraged us to explore the possibility of expanding the number of probes used within one hybridization. The high throughput capabilities of the PamChip™ should help laboratories to cope with the ever increasing number of genes which need to be screened for deletions or duplications.

P1053. A systematic mutation screening approach for syndromic and nonsyndromic forms of mental retardation in human Xp21.1-Xp11.23

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Apart from 7 syndromic forms of MRX which include Prieto and Renpenning, more than 20 non-syndromic MRX families can be mapped completely or partially to the Xp21.1-11.22 region. Therefore, we are establishing a detailed gene catalogue for a region flanked by markers DXS1237 and DXS146. This interval encompasses approximately 12 megabases and is under investigation via mapping and genomic sequencing at both the Sanger Centre (UK) and within our group. Exploring the genomic data, we have identified about 120 functional genes, including 65 known genes, 55 novel genes including transcripts with unknown function like e.g. the ones of the KIAA series, spliced ESTs and genes based on exon prediction only. In addition 31 pseudogenes have been found. Assuming that the region of interest is a representative part of the X chromosome due to its extreme variance in gene densities, we expect about 1000-1200 genes for the entire chromosome.
Of the 120 putative functional genes, 10 have been associated with genetic diseases, including one, TM4SF2, that is mutated in some families suffering from MR. Detailed expression studies, including in silico as well as wetlab experiments, are in process. Immobilization of the genes on membranes and subsequent RNA-hybridization approaches will allow the establishment of expression profiles in a wide variety of tissues. A systematic approach for mutation screening on the genomic and/or cDNA level has already been established and screening is underway for the Prieto and the Renpenning syndrome. Finally, cSNPs are being collected and documented.

P1054. ABI Prism® 3100 Genetic Analyzer: Further advances to expand productivity

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Applied Biosystems has always placed a large emphasis on continuous innovation. The 3100 system opened the doors for many researchers interested in speeding up their access to genetic information by providing a flexible platform that automated the process of genetic analysis. In order to keep pace with discovery, we have expanded this platform by further increasing the realm of possibilities, yet maintaining the quality and dependability one expects from Applied Biosystems. With the addition of the 80cm array for long read sequencing, and the 22 cm array for SNP analysis, we have increased the throughput of the instrument and allowed substantial cost savings to the investigator. When coupled with improvements to the Data Collection software the average investigator has acquired a more automated system, capable of allowing continuous operation for more than 24 hrs. We will discuss in detail how these features will expand your research capabilities and present a cost effective and handsfree solution, ready to decipher the unknown in molecular research.

P1055. Multiple testing in the survival analysis of microarray data

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DNA microarrays are increasingly being used to study the molecular basis of complex diseases like cancer, often aiming to identify genes potentially influencing important clinical outcomes (e.g. survival). To identify genes, survival is modeled (Cox proportional hazards) as a function of sets of gene expression levels. In its simplest version, the modeling is done one gene at a time. Prioritizing results for follow-up requires a realistic assessment of the significance of a relevant test statistic for the thousands of simultaneous comparisons made with a typical microarray.
We have studied the performance of several multiple testing procedures for survival data (including Bonferroni, Westfall and Young maxT family-wise error rate (FWER) controlling procedure, and Benjamini and Hochberg false discovery rate (FDR) controlling procedure). We illustrate their use on two publicly available cancer microarray datasets: a melanoma dataset with survival and gene expression measurements for 15 individuals on 3613 genes; and a lymphoma dataset with 40 individuals and 4026 genes. Although there are several unadjusted p-values smaller than 0.01, none of the genes appears particularly promising once adjustment is made for multiple testing. The lack of significant findings with either the FWER or FDR controlling procedures could be due to the small sample size, but also points to limitations of current approaches. Firstly, FWER controlling procedures may simply be too stringent in some microarray applications. Secondly, FDR controlling procedures, while less conservative than FWER controlling procedures, need to be refined to take into account the joint distribution of the gene expression levels.

P1056. Construction of a microarray on 5q31-q33 region to identify genes controlling resistance or susceptibility to parasitic diseases as schistosomiasis and malaria.

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Schistosomiasis affects 200 million people and is a major public health problem. Our group performed studies on the causes of high infections in an endemic area of Brazil. Certain subjects appeared to to be predisposed to high infections whereas others always exhibited low infection in spite of high exposure. This suggested that host-specific factors were important in the control of infection. Using segregation and linkage analysis, it was shown that there was strong evidence for the control of infection by a major locus (SM1, located in the 5q31-q33 region which contains a number of gene that encode cytokines that play an important role in the regulation of immune response against parasites. Immunological studies performed on the same population showed that SM1 control is linked to the differentiation of the T helper cells into Th1 or Th2 lymphocytes. Furthermore, it was also reported that blood parasitemie in Plasmodium falciparum are controlled by a locus located in the same region. This region is containing a large number of genes. In order to facilitate the analysis of this locus we have developed a systematic expression level analysis of the 5q31-q33 interval by microarray technology. The target will be a I.M.A.G.E. clone set arrayed on nylon support. The probe will be labeled with 33P. Indeed combination of nylon array with 33P labeled radioactive probes provides 100 fold better sensitivity, making it possible to perform expression profiling experiments using submicrogram amounts of unamplified total RNA from small biological samples.

P1057. Genome-wide survey of genes associated with coronary atherosclerosis and aortic aneurysm using polymorphic microsatellite markers and microarray technology

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The aim of this study is to exclusively identify genes related to coronary atherosclerosis and aortic aneurysm by genome-wide association analysis using polymorphic microsatellite markers. Microsatellite markers showed linkage disequilibria with disease-related alleles spanning from 100 to 200 kilobases (kb). For the purpose of genome-wide association studies, we finished identifying 30,000 polymorphic microsatellite markers from the human genome draft sequence which can cover whole genome. We then genotyped pooled DNA from 100 to 200 healthy Japanese individuals using those markers to confirm microsatellite polymorphism, allele frequency and heterozygosity. We have simultaneously started to apply those markers to conduct a genome-wide association study of atherosclerosis-susceptibility genes. We also investigated gene expression in aortic aneurysm tissue using GeneChip (Affymetrix) U95 sets. We report here our findings to date of different susceptibility locations within the genome. For example, we found strong association in the area from 7q32.3 to 7q36.3 confirmed by two different normal and diseased DNA pools. By narrowing the area, we believe there should be unknown gene related to coronary atherosclerosis. In addition, we found about 4700 known genes expressed in aortic aneurysm

tissue. Genes of osteopontin, apolipoprotein E, metalloproteinases and cathepsin D were highly upregulated. We believe the information gathered from DNA microarray technology would complement our genome-wide association studies. This combined approach should accelerate identification of atherosclerosis-related genes.

P1058. SNP analysis and linkage disequilibrium map of the human chromosome 22 using APEX arrays.

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Sequence variants in the human genome are responsible for the genetic component of disease, individuality and drug response. In order to find associations between SNPs and phenotype, large sample sets need to be genotyped with high-density markers. With chromosome 22 fully sequenced and a SNP map constructed across 22q, we have performed genotyping of Estonian and German samples and CEPH families with 1279 SNP markers. An array with 5200 oligonucleotides was designed to genotype each SNP twice from both DNA strands simultaneously using APEX technology. Allele frequencies, Hardy-Weinberg equilibrium and heterozygosities were calculated for each genotyped marker.
We have characterised the patterns of linkage disequilibrium and calculated D' for the whole 33Mb of chromosome 22. Our results demonstrated that along the chromosome the pattern of LD is highly variable, where regions of high LD are interspersed with regions of little or no detectable LD. It appears that SNPs from public databases will need additional testing in order to find out useful SNPs in respect of genotyping technology. Construction of LD maps across the human genome and identifying haplotypes in individual genomic regions will facilitate the identification and characterization of genetic variants responsible for common complex diseases.

P1059. MassARRAY™ Analysis of Fragmented Nucleic Acids: Applications in Typing, Sequence Validation, and Targeted SNP Discovery

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The MassARRAY™ System is widely used to do high-throughput scoring of single nucleotide polymorphisms (SNPs) using the MassEXTEND™ assay. A new application for high-throughput genetic analysis is presented which we call Fragmentation. Single-stranded nucleic acid is created and in four separate reactions fragmented at positions corresponding to each of the four bases. Using a reference sequence, such as the now available human genome, the precision, accuracy, and resolution of MALDI-TOF Mass Spectrometry allows one to definitively identify each resulting peak. Taken together, the collection of peaks creates a sort of biological barcode that supports high throughput typing. It also allows one to quickly and accurately perform sequence validation, key to many European patents. Recent software developments now also allow us to use this technology in high-throughput, targeted SNP discovery. In contrast to other techniques such as SSCP and dHPLC, in the majority of the cases Fragmentation not only detects SNPs, but can also definitively identify and locate the polymorphisms. This allows for the facile and quick design of a MassEXTEND assay for accurate, high-throughput SNP scoring.

P1060. Development and application of a cytochrome b SNP microarray to screen patients with mitochondrial complex III deficiency

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Complex III is the second enzyme in the electron transport chain of mitochondrial oxidative phosphorylation, and consists of several polypeptide subunits. One of these, cytochrome b, is encoded by mitochondrial (mt) DNA. So far, mutations in MTCYB and a nuclear encoded gene, BCS1L, have been found as cause of complex III deficiency. Patients with cytochrome b mutations may show widely varying clinical phenotypes, ranging from pure exercise intolerance to multisystem abnormalities, and the clinical findings also overlap with other mitochondrial diseases. These disorders are therefore best classified on the basis of pathogenic mtDNA variations. In order to systematically screen patients with biochemically determined or suspected complex III deficiency for single-nucleotide DNA variations, we have established a cytochrome b SNP microarray. This tool consists of covalently attached allele-specific primers representing all 90 SNPs and mutations of MTCYB found to date. In contrast to other SNP detection methods, only one PCR product comprising the complete MTCYB mtDNA and therefore all variations, is used as a target. The subsequent primer elongation protocol allows the typing of all 90 variations in one reaction. To test if this method is suitable for detecting heteroplasmy, we have mixed MTCYB DNA targets with known polymorphisms from two different samples. Material from approximately 70 patients is currently being analysed to detect novel cytochrome b phenotype-genotype correlations.
Our next aim will be the extension of this microarray to cover mtDNA encoded polypeptides of complex I.

P1061. Accurate SNP genotypes determined using a multiplex ligation/PCR based assay (OLA/PCR) in combination with a universal DNA microarray

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One of the expected consequences of the Human Genome Project is the practical utilization of extensive genetic diversity of populations. Public and private efforts have discovered polymorphic repeat sequences, as well as single nucleotide polymorphisms (SNPs) for genotyping. Several different databases now contain millions of candidate SNPs. In order to realize the full potential of SNP genotyping in large-scale population studies, sensitive, accurate and cost effective methods have to be established for scoring SNP genotypes. We have developed a ligation/PCR based assay (OLA/PCR) that uses a universal DNA microarray for SNP-specific assay product identification. Genomic DNA is interrogated with multiplex sets of ligation probes specific for selected SNP loci. After ligation, the resulting products are simultaneously amplified in a universal PCR reaction. The minimum single tube multiplex level has been shown to be ~50 loci, translating into a consumption of approximately 1 ng of gDNA per SNP genotype. We have assembled several multiplexes and tested these against *28* different CEPH DNA samples to demonstrate the feasibility of this approach. We will present data that describe the reproducibility and accuracy of this assay.

P 23. Therapy for Genetic Disorders

P1062. An anti-BRCA1 ribozyme act as a radiosensitizer in a melanoma cell line

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Background: Melanoma tumors are known to be radioresistant. Recent studies showed that *BRCA1* (BReast CAncer 1) is part of a multiprotein complex called BASC, involved in recognition and repair of aberrant DNA structures such as the double strands breaks induced by X-ray irradiation.
Aim of the study: to determine the role of *BRCA1* expression in the radioresistance of melanoma cell lines.
Materials and Methods: The A375 melanoma cell line was transfected with a vector expressing an anti-*BRCA1* ribozyme. Clones with diminished mRNA expression determined by real time quantitative RTPCR were irradiated and cell survival assays performed.
Results: Two clones, c88 and c91, exhibited a residual *BRCA1* expression of 60% and 50% respectively. Both had increased radiosensitivity compared to non transfected lines and lines transfected with the vector alone. Clone 88 was cultivated for two months, after which it recovered a normal *BRCA1* level of expression and interestingly also its radioresistance.
Discussion: Our data suggest that in melanoma as it has been shown in *BRCA1* mutated breast cell lines, *BRCA1* expression is involved in radioresistance. Work is in progress to study the pathways impaired after *BRCA1* down regulation using a multigenetic approach with DNA chips.

P1063. Bisphosphonate therapy for osteogenesis imperfecta

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Background: Severe osteogenesis imperfecta (OI) is a hereditary disorder characterised by increased bone fragility and progressive bone deformity. So far, no effective medical treatment is available. As secondary osteoporosis is an important feature of OI, antiresorptive activity of the aminobisphosphonates may improve clinical outcome in children.
Aim: to assess the clinical impact of the administration of bisphosphonates in Croatian OI patients.
Methods: We report results of 1-3 years treatment with intravenous pamidronate (APD) in seven children (four girls) of age 3 months - 11 years at entry, with severe OI. Pamidronate was administered in cycles as monthly infusions at a daily dose of 1-1,5 mg/kg during 6 months following pause for three months, or the same dose for three days every four months.
Results: Following treatment DEXA measurements showed a gradual increase in bone density in all patients. Number of confirmed fractures decreased in all. The reduction in pain and improvement in well-being and ability were impressive in two boys who had been confined to a wheelchair and now they walk using crutches. Acute phase reactions were noted during first infusion cycle in two children and asymptomatic hypocalcemia in three children. Three children gained excessive weight.
Conclusion: although bisphosphonates do not correct basic abnormalities in OI, they significantly alter the natural course of the disease and improve patients' quality of life. For the time being they seem not only effective but also devoid of any adverse effects on bone growth and remodelling.

P1064. Liposuction - a less invasive surgical method of debulking plexiform neurofibromas

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Neurofibromatosis type I (NF1) is a common autosomal dominant disorder in humans. The hallmark of NF1 is development of neural tumors. Plexiform neurofibromas are a major source of morbidity associated with NF1. These tumors are often large and inconveniently located, leading to disfigurement and compromise of vital structures by compression and tissue infiltration. Surgery still remains the only therapeutic option for patients on whom a tumor is causing disability or pain. In many patients tumors are not completely resectable and there is a high rate of tumor re-growth. Surgical removal of tumors is associated with high risk of damage of surrounding vital structures as well as with significant hemorrhage. Also, surgical debulking of tumors sometimes leads to extensive scaring which may be very disfiguring. We report a novel approach in surgical therapy of plexiform neurofibromas using liposuction in two patients. This method is less invasive than a conventional surgical tumor debulking. The procedure is well tolerated and post surgical recovery is short. Liposuction may be a prefered surgical method for debulking of superficial plexiform neurofibromas in patients with NF1.

P1065. In Vitro Modification Of Human Glioblastoma Cell Line Using Sfhr Technique

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Vascular endothelial growth factor (VEGF) is a positive effector of angiogenesis, encouraging neovascularization and growth of cancerous cells.We used an innovative gene modification approach based on Small Fragment Homologous Replacement (SFHR) technique for down-regulation of VEGF expression in human glioblastoma cell lines (U87). SFHR allows the modification of specific genes in situ by a gene targeting approach. The method is based on the introduction of small fragments of DNA into cells, that pair and replace the homologous endogenous sequence with the introduced fragment. We transfected U87 cells with a mutagenized DNA fragment homologous to exon 3 sequence of the VEGF gene. The corrective fragment was complexed with cationic liposome (Gene Porter, GTS) at different liposome to DNA charge ratio (+/-).The fragment was designed to insert a stop mutation in the wild type sequence in order to create a truncated and unstable VEGF protein. In addition, a unique Dde I restriction site was also inserted as a positive marker for replacement. Recombination at the appropriate genomic locus and expression of the modified CFTR mRNA were assayed using PCR amplification and restriction analysis. ELISA test was also performed to quantify residual VEGF protein secreted by cells. Molecular DNA analysis shows recombinant restriction pattern (DdeI) for lipid/charge ratio (+/-) from 42/1 to 2.7/1.The recombinant allele was absent at neutral charge of the complex.This study extends the efficacy of SFHR as a gene targeting techniques to introduce gene modifications able to inhibit expression of selected genes.
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P1066. Persistent failure of RNA/DNA oligonucleotides (chimeraplasts) in gene correction : targeting the HPRT gene

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Initial studies of gene correction using chimeric RNA/DNA oligonucleotides (RDOs), also named chimeraplasts, reported exciting rates of correction of point mutations (up to 40% in one in vivo model). However, the technique has not yet held its promises in the fields of transgenics and gene therapy, and some groups reported persistent failures.
In order to implement the technique in our laboratory, we targeted the HPRT gene. HPRT+ and HPRT- cells can be readily selected in HAT and in 6-thioguanine (6-TG) medium, respectively. The gene is expressed ubiquitously and is located on the X chromosome so that only one allele is to be mutated in male (XY) cells. We aimed at introducing a previously reported human mutation, which leaves virtually no residual enzymatic activity in functional assays. We introduced mutated and control RDOs in human male, diploid cell lines by transfection or direct microinjection. In all experiments we failed to observe any significant difference in the number of 6-TG-resistant clones between controls and RDO-treated cells. Moreover, resistant clones did not result from specific mutagenesis but merely from the background noise of the selection process.
Our experiments were designed to meet criteria defined to address criticisms expressed about early reports dealing with RDOs. In view of potential artifacts and the lack of reproducibility of published reports, we consider that conversion mediated by chimeric RDOs still awaits validation. We hope that confrontation of negative as well as positive results will help solving the problem of reproducibility of this potentially promising methodology.

P1067. PAC based engineering and expression of a genomic CFTR-GFP fusion gene.

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We engineered and initially analyzed expression of a 145 kb fusion gene. Starting from a genomic P1 based artificial chromosome (PAC) clone of the cystic fibrosis conductance regulator gene (CFTR) and the enhanced green fluorescence protein (EGFP) cDNA, a seven step cloning procedure resulted in a genomic CFTR-EGFP fusion construct (CG2). It contains approximately one half of the genomic CFTR gene locus from position -60 kb to exon 10, the EGFP cDNA replacing CFTR exons 10-24, and 2 kb of 3' sequences of the CFTR gene. Lipofection of the human lung sarcoma cell line HT1080 resulted in integration of one or possibly few copies of the construct into a host chromosome. Expression and correct splicing of the synthetic 10 exon gene has been confirmed by RT-PCR. Since expression of the primary transcript of CG2 requires intact

transfer of a minimum of 77 kb, the reporter should be useful for the development of large DNA transfer protocols. The reporter within it's own chromatin context now allows the analysis of transgene expression within target tissues

P1068. An engineered genomic CFTR-GFP fusion gene is expressed and correctly spliced on human artificial chromosomes.

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To assess co-lipofection of telomerized components cloned into a ditelomeric P1 phage based artificial chromosome vector (pTAT) as a means to incorporate genomic genes into human artificial chromosomes (HAC), we use our recently engineered, telomerized CFTR-EGFP fusion gene construct CGT21 (138 kb) which expresses a 77kb primary transcript under the control of the CFTR promoter. Comparably small amounts (100 ng) of CGT21 and telomerized alpha satellite DNA of chromosome 17 (a17T, 200 kb) where sufficient to generate a large number of co-transfected, blasticidin S resistant clones. Presence of the centromere efficiently changed the fate of the transferred gene from integration to HAC formation in 5 out of 5 lines analyzed, out of which 3 expressed and correctly spliced all 10 exons. Low copy HACs were detected by FISH in only 10-50% of metaphases, regardless whether on or off selection. Nevertheless, the majority (>87%) of single cell derived subclones which have been isolated after having been off selection for 30 generations, grew upon re-selection, indicating stable HACs in virtually all cells. While two of the lines presented stable HACs without integration after 60 generations, one HAC line showed an increasing proportion of metaphases (up to 40%) with integrations in two independent (painting probe) host chromosomes, indicating secondary events in this HT1080 cell clone. The data suggest that de novo HAC formation from naked DNA is an efficient means to stably transfer genomic copies of genes which can be engineered ad libitum in PACs.

P1069. Safety and efficacy of recombinant acid alpha-glucosidase (rhGAA) in patients with classical infantile Pompe disease

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Purpose: Pompe disease is an autosomal recessive muscle-wasting disorder caused by deficiency of the lysosomal enzyme GAA. Classical infantile Pompe disease is characterized by progressive cardiomyopathy, muscle weakness, respiratory insufficiency and death in early infancy. **Methods**: Evaluated are data of four patients with classical infantile Pompe disease participating in two separate Phase 2 open-label, multinational multicenter studies. Two patients are treated with rhGAA derived from milk of transgenic rabbits 40 mg/kg IV weekly and two patients with CHO-cell derived rhGAA 10 mg/kg IV weekly. Safety is evaluated by recording adverse events, vital signs, physical examination, antibodies to rhGAA and routine clinical lab tests. Clinical efficacy endpoints include ventilator-free survival, left ventricular mass, motor and cognitive development, and growth. Muscle biopsies are performed at baseline, 3 months, and 12 months. Two patients have been treated for more than one year and two for three months by now (mean age at enrollment 6,6 months, range 2,6-14,5). **Results**: Initial safety data indicates that the treatment is generally well-tolerated. There has been an overall improvement in left ventricular mass, cardiac function, skeletal muscle function and histologic appearance as evidenced by reduction of muscle glycogen. Younger patients and those with less advanced disease have shown the best clinical response. **Conclusions**: Our data suggest that rhGAA appears to be well-tolerated and capable of improving cardiac status and skeletal muscle function in patients with classical infantile Pompe disease. Further long-term safety and efficacy data are required to assess the potenial of this therapy.

P 24. Y chromosome, infertility

P1070. Yq microdeletions and male infertility in Iran

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Male factor is the cause of infertility in about 50 percent of infertile couples. 3-20% of infertile men with severe oligozoospermia or azoospermia have microdeletions of the long arm of Y chromosome (Yq) consistent with the location of AZF (Azoospermia Factor) in Yq11.23.
Recent Studies have shown the presents of 4 regions on the interval 6 of Y choromosome associated with male infertility. These are AZFa, AZFb, AZFc and AZFd which are involved in the process of spermatogenesis. DAZ and RBM are two multicopy gene families, which are expressed only in testis and have an important role in spermatogenesis. They are located at AZFc and AZFb respectively. Microdeletions in these regions cause severe oligozosspermia or azoospermia.
In this study DNA was extracted from blood lymphocytes of 120 azoospermic or severe oligozoospermic men in whom all known causes of infertility had been excluded. Twenty one Y specific STSs of deletion intervals 5 and 6 were selected for typing all subjects. Of the 120 oligo/azoospermic men, 8 (6.6%) had deletions of more than one STSs. All of the patients had microdeletions in AZFc (interval 6D), in which 75% of the cases had deletion in DAZ gene. Fifty percent of these individuals their deletion extended in AZFb region (Interval 6A).No microdeletions were detected in AZFa and RBM.

P1071. Clinical, cytogenetic and molecular genetic findings in patients with sterility or repeated pregnancy loss.

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In our study, cytogenetic and molecular genetic analyses were performed in 305 patients with sterility or repeated pregnancy loss from the Centre of Assisted Reproduction.
Cytogenetic examinations using karyotyping of peripheral blood were performed in all 305 individuals. Chromosomal abnormalities (mainly numerical aberrations of gonosomes, balanced translocations or inversions) were detected in about 10% patients.
DNA analyses were performed in two groups of 214 patients with reproductive failures. In the first group of patients (infertile men with oligo- or azoospermia) we performed molecular analyses of the Y by the PCR of sequences-tagged sites (STS-PCR) and mutation analyses of the most common mutations in the CFTR gene. In the second group of patients (positive family history or repeated pregnancy loss) we studied Leiden mutations of the factor V gene (G1691A) and the most common CFTR mutations. In all tested patients we diagnosed 7 carriers of CFTR mutations (dF508, dele 2,3(21kb), G542X), 7 carriers of Leiden mutations G1691A (1q23) and 4 men with the microdeletion of AZFc region on Yq chromosome. Our results of the complex screening programme in couples with sterility or repeated pregnancy loss from the Moravia region demonstrate chromosomal and / or gene disorders in 15,4% of patients.
In 8 patients with repeated IVF failures or spontaneous abortion we performed preimplantation genetic diagnosis (PGD) of numerical abnormalities for chromosomes 13, 18, 21, X and Y using fluorescence in situ hybridization (FISH). Two of the women have become pregnant after PGD.

P1072. Molecular detection of Y chromosome microdeletions: a Slovak study.

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Male factor infertility accounts for about half of the cases of couple infertilty. Microdeletions of the long arm of the human Y chromosome are associated with spermatogenic failure and have been used to define three regions of Yq (AZFa, AZFb, AZFc) that are recurrently deleted in infertile males. Several genes have been identified within this region and have been proposed as candidates for infertility. About 10-15% of azoospermic and about 5-10% of severely oligospermic men have Yq microdeletions. The deletions are associated with a wide range of histological pictures ranging from Sertoli cell only syndrome to spermatogenic arrest and severe hypospermatogenesis. Assisted reproduction techniques such as in vitro fertilization and Intra Cytoplasmic Sperm Injection alone represent an efficient therapy for these patients. However the potencial of these techniques to transmit genetic defects causing male infertility raises the need for a systematic genetic screening and genetic counselling of these patients.
We have investigated 168 infertile men (67 with azoospermia, 61 with oligoasthenospermia, 26 with oligospermia and 11 with other diagnosis). For each patient, five multiplex PCR analyses were performed on DNA isolated from leukocytes derived from peripheral blood to screen 10 sY- sequences on Yq. Microdeletions were detected in 11/168 (6,5%) infertile men. Seven of the 67 azoospermic men (10,7%) had such deletions. Of the 61 oligoasthenospermic men 2 (3,2%) had deletion, 1 of the 26 oligospermic man had deletion, and among other diagnoses microdeletion was detected in 1 man.

P1073. Molecular analysis of the Yq microdeletions in male with azoospermia and oligospermia and in children of liquidators of Chernobyl accident consequences from Ukraine.

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Male infertility is caused by many different exogenous and endogenous factors. In addition to chromosomal anomalies, microdeletions in the azoospermic factor region (AZF) in the long arm of Y-chromosome have been detected in men with azoospermia or sever oligospermia. We have screened 105 men with azoospermia and oligospermia - patients of "ISIDA-IVF" clinic involved in ICSI (intracytoplasmic sperm injection) program as well 70 boys - sons of fathers-liquidators (Chernobyl eccident clean-up workers). Our study consists of 11 primer pairs that are homologous to previously identified and mapped sequence tagged sites (STS). The STS primers tested on each subject were sY84, sY85 (AZFa); sY117; sY124, sY134 (AZFb); sY141, sY146; sY240, sY254 (DAZ), sY255 (DAZ), sY158 (AZFc). The samples were analysed for Y-chromosome microdeletion by multiplex-PCR. The PCR products were analyzed on a 1,8% agarose gel. SRY was used as internal controls of PCR reactions. Any de novo deletions was not found in the group of liquidators children. In five of the 105 infertil men (4,76%) it was shown 1 deletion of at AZFb (0,95%) and 4 deletions of at AZFc (gene DAZ) (3,81%) regions prevailing which majority is concentrated in AZFc region. During patients research we have made a conclusions: a) the damage of genes from AZFb region results in impairments of last stage spermatogenesis; b) deletions in AZFc region are critical for early stage spermatogenesis. The genetic consultating and preimplantation sex analysis were recommended for the patients with found deletions.

P1074. Dicentric chromosome (Y;22) resulting in a microdeletion encompassing SHOX in a boy with short stature and no dysosteochondrosis.

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We report a 4 year-old child with short stature, and a dicentric chromosome with a deletion of the distal part of chromosome Yp. The parents were not consanguineous, and did not have any relevant history of genetic disease. The pregnancy was uneventful, until intra-uterine growth retardation was noted. Prenatal karyotyping showed a (Y;22) translocation. No structural fetal abnormality was shown at ultrasound examination, and the pregnancy went to term. A growth retarded boy with an otherwise normal physical examination was delivered at 39 weeks. At age 4, the child had short stature (-3SD) without mental retardation. Radiological examination of the wrist was normal. Blood karyotyping confirmed the chromosomal rearrangement. C-banding showed a dicentric chromosome, and FISH with centromeric probes confirmed the presence of both chromosome Y and 22 centromeres on the derivative chromosome. FISH using a TUPLE1 probe did not show any rearrangement at 22q11 or at the ARSA locus. Using a subtelomeric probe, a deletion of the distal Yp region was shown, whereas SRY was not deleted as shown by FISH. A set of two probes encompassing the SHOX region were used for FISH and demonstrated a deletion of this gene. The karyotype was thus 45,X,der(Y;22)(p11;q11).ish del(Y)(p11p11)(SRY+,SHOX-).
Haploinsufficiency of SHOX may result in isolated growth retardation or in Leri-Weill dysostosis, which is not an obvious diagnosis in our patient, but may appear at a later age. Although other loci may have been deleted, no other genes seem to have resulted in haploinsufficiency in our patient.

P1075. Cytogenetic Analysis And Y Chromosome Microdeletion Results In Infertile Males Undergoing Assisted Reproductive Technology (ART)

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Infertility affects approximately 15% of all couples. Among these couples male factors are the aetiology in about 50% of cases. Besides many different factors in male infertility, chromosomal aberration and Y chromosome deletion screening became one of the standart procedures of ART clinics.
We report the cytogenetic and chromosome microdeletion results of 315 men with azoospermia or severe oligospermia. After clinical examination, seminal fluid and hormonal tests, cytogenetic investigation was performed by GTG-banding. The screening of microdeletions in 18 loci of the Y chromosome was performed by multiplex polymerase chain reaction. In 16 months we have performed 315 semen analysis according to WHO guidelines in 157 azoospermic (50%) and 158 oligospermic (50%) patients. Cytogenetic analysis was performed in 256 cases of the group and abnormality detected in 26 (10,2%) patients including 12 translocations, 8 Klinefelter syndromes, 3 invertions, 1 marker chromosome 15 carrier, 1 47,XYY, 1 mosaic 45,X(8%). Microdeletions of AZFa, AZFb, AZFc and AZFd regions of chromosome Y were detected in 1, 6, 11 and 11 patients respectively. Frequency of abnormal karyotype (10,2%) and frequency of Y microdeletions (4,1%) suggests the need for genetic testing of ART candidates and the requirement for genetic counselling.

P1076. Partial deletions of the DAZ gene cluster in males with idiopathic infertility

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About 10% of infertile males show microdeletions of the Y chromosome, the majority of which involving the AZFc locus. The DAZ gene maps within AZFc with four copies (DAZ1-4) and is considered as the major candidate for infertility. Since the PCR approach used for the screening of the Y chromosome can detect only deletions of all the DAZ copies, so far it is not know whether or not partial deletions of the cluster are related to a spermatogenesis failure. We studied the presence of partial deletions of the DAZ gene cluster in 42 infertile patients and in 67 fertile controls. This study was based on the presence within the DAZ sequence of three intronic SNPs able to distinguish among the four gene copies. The STSs sY581, sY586 and sY587 were PCR amplified and digested with Sau3A, Taq I and Dra I restriction enzymes, respectively. Absence of the band specific for DAZ2 was evidenced in 13 patients (30.9%) and in 10 controls (14.9%). Thus, the absence of DAZ2 appear compatible with normal spermatogenesis, but is two times more frequent in infertile males, suggesting that this condition could make patients prone to other genetic or environmental factor able

to reduce their fertility. On the other hand, the loss of DAZ1-DAZ4 and DAZ2-DAZ3 was detected only in 4 patients, but never in controls, suggesting that this condition is not compatible with normal spermatogenesis. Experiments with Fiber FISH analysis are in progress in order to confirm these results with a different approach.

P1077. An Inherited Three Base Pair Deletion In A Sp1 Binding Site In The 5' Non-coding Region Of Sry Gene Is Associated With Sex Reversal

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The condition named 46,XY pure gonadal dysgenesis is characterized by a female phenotype with full development of unambiguous female genitalia, normally developed Müllerian structures and streak gonads. Male to female sex reversal in 46,XY individuals results from failure of testis development and is presumed to be due to mutations in the SRY gene or in other genes involved in the sexual differentiation pathway. The majority SRY mutations described so far were found within the coding region, mainly in the HMG-box conserved domain. We have tested a female patient with 46,XY pure gonadal dysgenesis for mutations in the SRY gene. SRY complete open reading frame and 380 pb of the 5' flanking region were amplified by PCR and directly sequenced. Sequence analysis revealed no mutations within SRY coding region. However, a three base pair deletion was found in the 5' flanking sequence. This deletion involves a Sp1 binding site motif and is located next to a potential WT1 responsive element. This is the first report of a mutation in these putative SRY regulatory elements associated with sex reversal. Familial investigation revealed that the father bears the same deletion and two cousins were referred to have sexual ambiguity. Polymorphism was discarded, as it was not found in unrelated males. Different patient studied previously had the R30I familial SRY mutation within a phosphorylation site and it is associated to pure and partial gonadal dysgenesis as well as to normal phenotype. Those two mutations might influence sex determination depending on the in vivo environment.

P1078. The Leri-Weill and Turner-Syndrome homeobox gene SHOX encodes a cell-type specific transcriptional activator

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Functional impairment of the human homeobox gene SHOX causes short stature and Madelung deformity in Leri-Weill syndrome and has recently been implicated in additional skeletal malformations frequently observed in Turner syndrome. To enhance our understanding of the underlying mechanism of action, we have established a cell culture model consisting of four stably transfected cell lines and analysed the functional properties of the SHOX protein on a molecular level. Results show that the SHOX encoded protein is located exclusively within the nucleus of a variety of cell lines, including U2Os, HEK293, COS7 and NIH3T3 cells. In contrast to this cell-type independent nuclear translocation, the transactivating potential of the SHOX protein on different luciferase reporter constructs was observed only in the osteogenic cell line U2Os. Since C-terminally truncated forms of SHOX lead to Leri-Weill syndrome and idiopathic short stature, we have compared the activity of wild-type and truncated SHOX proteins. Interestingly, C-terminally truncated SHOX proteins are inactive with regards to target gene activation. These results for the first time provide an explanation of SHOX related phenotypes on a molecular level and suggest the existence of qualitative trait loci modulating SHOX activity in a cell-type specific manner.

P1079. The Y chromosome microdeletions in Czech men with severe reproduction disorders

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The aim of the study was the ascertainment of the Y chromosome microdeletions frequency in 197 Czech men with severe reproductive disorders, characterised by different types of abnormal sperm counts (SC) and morphology, referred for reproductive genetic counselling. For Y chromosome microdeletions detection multiplex PCR with amplification of STS markers for AZFa (sY84, sY86), AZFb (sY127, sY134), AZFc (sY254, sY255) and ZFY and SRY was used. Microdeletion frequency was analysed in four groups of males according to their sperm count: I. (azoospermia - 64x), II. (SC<1x106/ml - 19x), III. (SC 1-20x106/ml - 101x), IV. (SC>20x106/ml - 12x). The Y chromosome microdeletion was found in 8/197 (4.1%) examined males. Nevertheless, these microdeletions were detected in azoospermic males only in 7/64 (10.9%). The AZFc (sY254, sY255) deletion was disclosed in 4/64 (6.3%), AZFc (sY254, sY255) deletion combined with AZFb (sY127, sY134) deletion in 2/64 (3.1%) and AZFc (sY254, sY255) deletion combined with AZFb (sY134 only) deletion in 1/64 (1.6%). Microdeletion of AZFc (sY254, sY255) was also disclosed in one man referred with necrospermia without an exact sperm count. The 10.9% frequency of Y chromosome microdeletions in Czech azoospermic men does not differ from so far as reported range of its prevalence and no AZFa deletion was found.
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P1080. Number Of Ctg Repeats In Dmpk Gene And Male Infertility

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BACKGROUND. The clinical picture of trinucleotide repeat diseases: myotonic dystrophy type 1 (DM1), fragile XA syndrome (FraXA) and spinobulbar muscular atrophy (SBMA), is manifested as reduced infertility in men. The principle feature in adults with severe form of DM1 and SBMA is testicular atrophy, and for FraXA syndrome macroorchidism is characteristic. Several studies have reported that fertile males with a higher number of CAG repeats in normal range of androgen receptor have an increased risk of impaired spermatogenesis. In DM1 hypergonadotropic hypogonadism, leading to azoospermia and impotence, is frequently observed. An increased number of CTG repeat in infertile males could represent a risk of DM1 among offspring conceived by intracytoplasmic injection of sperm (ICSI).
The aim of this study was to compare the distribution of number of CTG repeats in normal range between infertile and fertile group of males to evaluate the involvement of CTG repeat number in male infertility.
PATIENTS. The number of CTG repeats in 107 infertile men (38 with azoospermia and 69 with oligoasthenoteratozoospermia), ICSI candidates, and in 102 fertile men with no sign of myotonia or muscle weakness was determined.
RESULTS. Among infertile males no premutation or mutation carriers were found. The distribution of the number of CTG repeats compared between infertile and fertile males was not statistically significant ($p = 0.825$).
CONCLUSION. We conclude that the number of CTG repeats in normal range does not contribute to male infertility. Moreover, this results show that increased risk of DM1 among offspring conceived by ICSI is unlikely.

P1081. Risk of sex chromosome mosaicism associated with Y chromosome AZFc microdeletions

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Deletions of specific Y chromosome regions cause male infertility. Recent advances in infertility treatment permits deleted Y-chromosomes to be transmitted to male offspring with the assumption that there will be no clinical consequences other than infertility in adult life. Recent data suggested that sex chromosome mosaicism could arise when AZFc-deleted Y chromosomes are transmitted from one generation to another by ICSI. If this hypothesis is correct, it raises the possibility that some patients with 45,X/46,XY karyotypes, such

as patients with Turner-related phenotypes or sexual ambiguities may in fact harbour Y chromosome microdeletions. To test this hypothesis, we screened a group of patients with a 45,X/46,XY karyotype for Y microdeletions using several Y-specific markers. We show that 33% of patients presenting with Turner stigmata and/or sexual ambiguities and a 45,X/46,XY karyotype, carried Y chromosome microdeletions of distal Yq. These results highlight a potential risk for offspring born to fathers carrying Y chromosome microdeletions and treated by assisted reproductive techniques. The risk is the development of sex chromosome aneuploidy during foetal and embryonic development. The clinical consequences of this can be severe, including Turner syndrome, mixed gonadal dysgenesis, male pseudohermaphroditism and rarely mild mental retardation or autism. Our data may also explain in part the observation that in almost three-quarters of all 45,X patients with Turner syndrome, the X chromosome is maternal in origin. Concluding, the transmission of Y chromosome microdeletions could potentially have more severe clinical consequences other than male infertility, such as the development of sexual ambiguities and/or Turner stigmata.

P1082. A national based population screening for AZF deletions in patients with severe spermatogenic failure and normal fertile controls, using a specific study and experimental design

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Y chromosome microdeletions in the azoospermia factor (AZF) locus have been associated with spermatogenic failure. The frequency of AZF deletions is estimated to be about 7.3% in infertile men, although, in the literature the frequency varies between 1% and 55.5%. Therefore, considerable debate remains concerning the frequency, the position, and the phenotypes associated with Y chromosome microdeletions. In order to elucidate the above debate, we designed a national based population screening with well-defined study and experimental criteria. Eighty Greek-Cypriot patients that met the selection criteria were included in this study as well as 50 normal controls with proven fertility. All samples were collected from all districts of the island of Cyprus as the population is of the same religious, geographic and ethnic origin. All patients and controls had detailed clinical information and at least two semen analysis reports based on WHO standards. Samples with abnormal karyotypes, obstructive azoospermia or oligospermia with >2 X 106/ml were excluded from this study. The experimental design required a referral team and laboratory to undertake the responsibility, collect all samples, all clinical and laboratory information, isolate DNA and carry out all tests, data analysis and interpretation. This study showed that Y chromosome microdeletions are specific for spermatogenic failure. Under the specific patient selection criteria and experimental design the overall frequency is 6.3% or 12.5% among patients with azoospermia and 2.1% in oligospermia (<2 X 106/ml). The variation in deletion frequency reported in other studies is probably attributed to the patient selection criteria and experimental design.

P1083. Polymorphism of the MUC 1 mucin 60-bp microsatelite is not associated to women infertility nor to embryo implantation failure

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Recent studies have demonstrated that the transmembrane mucin glycoprotein, Muc-1, is abundantly expressed at the apical surface of luminal epithelia under most conditions and is invariably reduced in receptive uteri. These and other observations have led to the suggestion that mucins serve an antiadhesive role and function to maintain a nonreceptive uterine state. A polymorphic variation of the MUC 1 gene (Horne et al., 2001 - Lancet, vol. 357, no. 9265) has been associated to women infertility due to suspected failure of embryo implantation, based on the significant greater size of the lower allele observed in the infertile group. Our aim was to confirm this preliminary data through a long-PCR methodology, based on primers flanking the 60-bp polymorphic microsatelite associated to the binding domain of the Muc-1 glycoprotein. DNA samples were obtained from twenty-one women patients, 10 fertile and 11 infertile (three or more implantation failures), and were amplified in long-PCR conditions using the Platinum Pfx DNA polymerase. Amplification generated fragments sizes from 1.6 to 2.9 kb. The average size for the lower allele was 1.68 kb for both groups, and for the upper allele was 2.29 and 2.47 kb (P>0.05), respectively for fertile and infertile groups. In conclusion, the 60-bp microsatelite polymorphism of MUC 1 gene was not associated to women implantation failure.

P1084. Y chromosome microdeletions in infertile azoo- and oligozoospermic men in Russia

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Infertility affects 15% couples and in 50-60% of these cases qualitative and/or quantitative abnormalities of sperm are found. About in half of these cases the etiology of male infertility, defined as idiopathic, may be associated with genetic factors. Major known genetic cause for male infertility is Y-microdeletions in AZF locus (Azoospermia Factor) that contains three non-overlapping Yq11 regions (AZFa, AZFb and AZFc). Several genes, have been identified within these regions, are considered as candidates for male infertility. AZF-microdeletions are associated with spermatogenic failure ranging from Sertoli Cell Only Syndrome (SCOS) to spermatogenic arrest and severe hypospermatogenesis.
We have studied cohort of infertile men with azoospermia and severe oligozoospermia. Clinical examination, seminal fluid and cytogenetic analysis was carried out on 89 males. Cytogenetic investigation was performed according standard methods (GTG-banding). No chromosomal anomalies were found in men. Molecular analysis was carried out using leucocyte DNA by multiplex PCR. Primer set contains 9 Y-specific pairs: SRY; ZFY; sY84, sY86, sY615 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). Eight markers were selected on "Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions" (Simoni et al., 2000), and one additional marker sY615 was used for AZFa subregion. Sequences of all primers are original. Microdeletions have been found in 13 cases. Eleven patients had microdeletions in AZFc (9 with azoospermia and two - severe oligozoospermia), 2 patients had microdeletions AZFb+c and azoospremia. The frequency of microdeletions in this study is 14.6% that is consistent with published data.

P1085. FOXL2 mutations in syndromic and non-syndromic female infertility

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Type I Blepharophimosis/Ptosis/Epicanthus inversus Syndrome (BPES) is an autosomal dominant disorder in which eyelid abnormalities are associated with Ovarian Failure leading to Female Infertility. Apparently, type II shows only the eyelid defects . We recently cloned a novel gene, FOXL2, belonging to the family of the winged helix/forkhead transcription factor, that we found to be mutated in both types of BPES.
We carried out a FOXL2 mutation screening on 45 BPES families. On all 13 type I BPES families analyzed we identified nonsense and frameshift mutations. In 7 type II BPES patients we observed apparently milder mutations: a 30 bp in-frame duplication (909_939dup) present in 3 independent cases, 3 different missense mutations and a 3 basepair deletion. We detected FOXL2 mutations in 7 additional BPES patients whose clinical type could not be determined. These results suggest a genotype-phenotype correlation ; FOXL2 loss-of-function mutations are probably incompatible with any kind of female fertility while milder mutations could allow a child-bearing activity but nonetheless affect ovarian function. For example,

the 909_939dup was present in a girl with overt Ovarian Failure whose affected mother also had a history of Premature Ovarian Failure (POF) . A position effect for the FOXL2 gene or mutations in another gene could be responsible for those cases in which we found no mutations. In addition, we are concluding the screening of FOXL2 for 100 POF patients, and to now we only found one missense mutation in a patient in the coding region of the gene.

P1086. Microdeletion analysis of the AZF region in Male Infertility

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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 and are recurrently deleted in infertile males. 102 infertile males were included in this study. Semen analysis was done in each case to determine the spermatogenic status. They were subjected to detailed clinical examination, endocrinological and cytogenetic study.In all cytogenetically normal cases (n=70) microdeletion analysis was carried out using PCR. For this genomic DNA was extracted using peripheral blood. The STS primers tested on each subject were sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc).Eight of the seventy cases (11.4%) showed deletion of at least one of the STS markers. Four cases had AZFc deletion, three cases had AZFa and AZFb deletion and one case showed AZFb deletion alone. The 3 cases with AZFa and AZFb deletions had Sertoli Cell Only Syndrome (SCO). Two of the three cases with AZFc deletion had hypospermatogenesis and in the third case there was maturation arrest. In two cases with AZF deletion FNAC was not possible as they were cryptorchid. Thus cases with AZFa and AZFb deletion have poor prognosis and cases with AZFc deletion can go in for Assisted Reproductive technology as there testis show spermatogenesis though at a reduced rate. Therefore in all cases of idiopathic infertility a genetic aetiolgy should be established to provide the most adapted therapeutics to the infertile couple.

EMPAG Abstracts

EMPAG Plenary Sessions

E-PS 1. Psychosocial Impact of Genetic Disease

E-PS01. Living with Marfan syndrome; the European experience.

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Living with Marfan syndrome; the European experience.
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A specialised questionnaire, containing 88 multiple choice questions on medical and psychosocial aspects, was sent to patients with Marfan Syndrome (MFS) from 7 European countries. We present data from 857 individuals on the quality of life and the psychosocial well being as experienced by persons with MFS. A scoring system was established to assess the objective severity of the condition in each individual. The subjective severity that stands for the patients' own perception of their condition was questioned. We compared the results with an objective coping score based on questions relating to the psychosocial adjustment. The data show that MFS represents a significant burden on many aspects of daily life but that most individuals are coping effectively with the disorder. The level of coping and the quality of life is determined by the subjective rather than by the objective severity. The subjective attitude towards the condition influences the attitude towards professional activities, relationships and reproductive options. Many individuals have difficulties discussing problems associated with the MFS. It remains important to stimulate the support of family, friends and patient support groups since social isolation negatively correlates with depression and anxiety. Professional support must pay attention to the threat of social isolation. We conclude that creating a positive frame of reference is an important element for their psychosocial well being.

E-PS02. Living with FAP in the family

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Objective: Predictive genetic testing for familial adenomatous polyposis (FAP) is applied for nearly ten years, but little is known about its psychosocial effects. The aim of the study presented here is to investigate long-term psychological and social consequences of living with the knowledge of FAP running in the family. The main interest is to analyse the influence of genetic knowledge concerning reproductive decisions.
Method: 16 affected people from all over Germany were asked to tell their life-histories in order to investigate how people suffering from FAP live with their genetic knowledge. The qualitative evaluation of transcripts of the interviews followed the methodology of narrative biographical analysis developed by G. Rosenthal (1995).
Findings: All people investigated have learned to manage their own disease during their disease trajectories and to get over the uncertainties concerning their own health. However, the possibility to pass on or to have passed on the mutated APC-gen to the children remains a steady cause of uncertainty which is a major problem living with FAP. Four different types of strategies to cope with this uncertainty were found which have different consequences concerning reproductive decisions or explaining facts of FAP to the children. Due to the biographical approach of the analysis it is shown that the coping strategies mainly depend on individual experiences rather than on rational considerations concerning the genetic disease.
Conclusion: The individual case history has more influence on reproductive decisions and health care behaviour than rational genetic considerations. This must be considered in counselling models.

E-PS03. The Psychosocial Aspects of Skeletal Dysplasia and the Impact of Molecular Genetic diagnosis-An Exploratory Study

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Aim of this study was to explore the mental health and psycho-social problems associated with an undiagnosed skeletal dysplasia and the impact of a precise molecular diagnosis, which is hypothesised to have a significant psychological benefit for both the individuals and their families. Definitive diagnosis removes uncertainty, allows risk assessment and the possibility of testing for other family members. Previous research has explored the psychological and mental health aspects of having a skeletal dysplasia and has highlighted a range of difficulties particularly in adolescents. (Apajasalo et al., 1998; Hunter, 1998 and Vallmitjana, 1999). However, these studies relied upon quantitative measures. A qualitative methodological approach is effective in giving a fuller picture of the subjective experiences. A pilot sample consisting of both adolescents, older adults and parents (n=19) were interviewed using the constant comparative method with theoretical sampling derived from Grounded Theory (Glaser and Strauss, 1967; Strauss and Corbin, 1998) in order to identify key areas of concern. Analysis was performed using Atlas.ti software based on grounded theory principles, effective for qualitative analysis. Three major categories of themes were elicited. Firstly, the effect of the disorder on the psychological and social well-being of the individual when living with a conditon of this nature. The other two major themes concerned aspects of medical care and issues surrounding genetic diagnosis and testing. The data suggests that both psycho-social and practical benefits and problems are associated with molecular diagnosis for the patients and their families, important for the future direction of healthcare service provision.

E-PS04. Living with Achondroplasia in an Average-Sized World

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Mutations in the gene encoding fibroblast growth factor receptor 3 cause achondroplasia, the most common form of inherited dwarfism. Although there are 10,000 individuals with achondroplasia in the United States, there has been little study of the quality of life of individuals living with the condition. For this study, surveys were collected from 189 individuals affected with achondroplasia (ACH) and 136 relatives of average stature (FDR). FDR and ACH individuals differed significantly in their perception of achondroplasia as well as in their evaluation of advantages and disadvantages that accompany the condition. Overall scores for quality of life (QOL) as well as scores in each of the four subdomains were significantly lower in affected individuals than in the relatives. Lower self-esteem scores, affected status, and lower income were the most significant predictors of a reduced quality of life index. A qualitative analysis of open responses to questions about the advantages and disadvantages of achondroplasia revealed that individuals were as likely to cite disadvantages relating to their health and functioning as they were to cite social disadvantages. Affected individuals' reduction in quality of life, lower than that which would be expected by relevant sociodemographic variables, can be interpreted to be a result of negative factors relating to social constraints on living in an average-sized world. Genetics professionals should consider a broad conception of quality of life as reported by individuals themselves to better understand and inform others about disabilities.

E-PS 2. Predictive Testing for Late Onset Disease

E-PS05. Impact on perceived control and risk-reducing behaviour of genetic testing for Familial Hypercholesterolaemia (FH): a randomised controlled trial

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Background There are concerns that predictive genetic testing may reduce perceived control over a disease, thereby reducing motivation to engage in risk-reducing behaviours.
Purpose To test the hypothesis that a diagnosis of familial

hypercholesterolaemia (FH) incorporating genetic testing reduces perceptions of control over FH and adherence to risk-reducing behaviour.
Method
340 families (340 FH probands and 129 adult relatives) were randomised to: Routine clinical diagnosis; or Routine clinical diagnosis plus genetic testing (mutation searching in patients and direct gene testing in relatives). Outcomes were assessed at baseline, one week and six months after diagnostic testing.
Results
81% of probands and 73% of relatives completed the trial. Mutations were found in 29% of the 196 probands undergoing mutation searches and in 46% of the 37 relatives undergoing direct gene testing. The diagnosis of FH where a mutation was found had no impact on perceived control or adherence to risk-reducing behaviour at either time point, compared with a non-genetic diagnosis of FH. By contrast, for those in whom a mutation search was unsuccessful, perceived control over FH and heart disease was lower one week after testing, but not at six months.
Conclusion
The hypothesis was not supported. Future studies are needed to determine whether similar results are obtained in general populations undergoing genetic testing to learn of increased risks for early heart disease and other conditions.

E-PS06. Parents' responses to genetic testing in their children for long QT syndrome

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Purpose: To assess the psychological reactions displayed by parents who applied for genetic testing of their children before and after disclosure of genetic results for inherited Long QT Syndrome.
Methods: Subjects are parents(n = 41) who applied for genetic testing of their children less than 16 years old. Before and two weeks after DNA test disclosure these parents completed questionnaires that assess levels of anxiety related to the test disclosure (IES), depression (BDI) and general anxiety (STAI).
Results: All parents showed high levels of distress at predisclosure measurement. After disclosure, parents of carrier-children (n = 24) had significantly higher scores on the IES (t = 5.03, P = .000), the BDI (t = 2.65, P = .013) and on the STAI-s (t = 2.34, P = .031) compared with parents without a carrier-child (n = 12). After disclosure, the percentage of parents of child-carriers with high or very high scores in comparison with the normal population on the distress measures is considerable: 41 % had IES scores indicating a traumatic impact, 29 % of the carriers had BDI scores indicating depression and 53 % had STAI scores indicating a high level of general anxiety.
Conclusion: High levels of psychological distress in our group may reflect great suffering for having a child with a life-threatening inherited arrhythmia. Whether the high levels of distress in these parents can be valued as a sign of maladjustment is dependent on the outcome of a follow-up study.

E-PS07. Interactional framing of decision-making and coping trajectories in counselling for predictive testing for Huntington's Disease

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Genetic professionals and clients in Huntington's clinics may assign different meanings to the extended format of the counselling protocols for predictive testing. While counsellors typically see their role as following a specific agenda for the evaluation of clients' understanding of predictive testing, including possible results and their implications for families, clients may regard their experience of protocols as of a gatekeeping nature that need to be complied with to gain access to testing.
We use discourse analytic methods to examine the interactionally complex framing of clients' decision-making and coping trajectories, as prompted by counsellors' agenda-driven probing. The notion of frame is taken from Goffman (1974:21) to denote `schemata of interpretation' that enable participants`to locate, perceive, identify, and label' occurrences and events within their everyday life spaces. The data includes over 40 detailed transcripts of audio-recorded pre-test sessions for HD involving 15 families in Wales. Our analysis suggests that the pre-test counselling sessions are predominantly oriented towards a display of clients' (i) accounts of the trajectories that underpin their current decisions about having or not having a predictive test, and (ii) reflections on how they are prepared to cope with the future implications a positive or negative test result may have upon themselves and their families. The analytic focus will be the incidences of misalignments in counsellors' attempts to facilitate clients' accounts of decision-making and coping - both retrospectively and prospectively - and in clients' differential orientations to the temporal dimensions of their experience of living with the genetic condition.

E-PS08. Test motivation, predictive test result for Huntington's disease and the evolution of psychological distress over a five year period.

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The aims of the present paper are: (1) a description of general and specific distress in tested persons five years post-test, (2) an assessment of the evolution of depression level, anxiety and ego-strength over time, in function of test result and (3) the role of test motivation in explaining interindividual differences in psychological distress over time.
Methods: The 5-year assessment is an extensive psychological evaluation of tested persons (24 carriers, 33 non-carriers), using psychometric tests and qualitative measures. Some of these tests had also been administered at baseline and 1 year post-test.
Results: Carriers did not differ from non-carriers with regard to general distress. With regard to specific distress, carriers had significantly more avoidance behaviour and had less positive feelings about their test result than non-carriers five years post-test. The study further showed that mean depression level, general and HD-specific anxiety had significantly decreased at the 5 year assessment, compared with the baseline level. The evolution did not significantly differ for carriers and non-carriers. We found clear evidence for the role of the participant's test motivation in psychological distress. Persons who asked the test to get rid of the uncertainty, without specifying implications for substantial life areas, had more psychological distress before and after the test, compared to those who wanted the test to take action in an important life domain. Moreover, the pattern of distress differed over time, depending on the test motivation.
Implications of the findings for pre-and post-test counselling will be discussed.

E-PS09. Damned if you do, damned if you don't: the role of religious faith in predictive and diagnostic testing for Huntington's disease

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Presymptomatic predictive testing for Huntington's disease (HD) has been provided for those at risk of this neurodegenerative disease for more than 15 years at centres throughout the world. Professionals from the international community, with family members, created a code of practice which is adhered to in most centres world wide. Despite cultural differences, this protocol has been remarkably successful in ensuring support for those who seek to know whether or not they have the mutation for HD. Guidance for the test suggests pre-test discussion ought to take place about issues involving family relationships, potential insurance problems, and employment difficulties, but religious faith is generally not included as a discussion topic. The role of spirituality in health and well being has been extensively explored in the medical literature, and in the field of prediction for cancer risk. Religious faith has been noted to be important in providing support after a predictive test result for HD, but there is no discussion about the role of religion in the decision making process. This paper will discuss the literature and give four

case histories where issues surrounding religious faith have caused significant difficulties for the individuals who underwent predictive or diagnostic testing whether or not favourable or unfavourable results were produced.

E-PS 3. Different approaches to genetic counselling and psychosocial service provision

E-PS10. Provision of Genetic Services in Europe: Do we meet the Community Needs?

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Genetics and biotechnology provide us with new diagnostic tests (around 600 genetic diseases can be accurately diagnosed) and much needed therapeutic tools (around 100 diseases are now treated with a specific drug) , but health technology, practices and procedures cannot be left to the vagaries of economic forces and personal interests. There appears to be tendency to adopt the new applications of molecular genetics without always providing appropriate information and counselling. There are profound economic and technological inequalities between countries and population groups within Europe. Efforts must be made to propose and harmonize safeguards so that such inequalities are not aggravated, that the safety and rights of all individuals and communities are adequately protected.
The rapidity and complexity of the progress made in the field of human genetics generates the need to evaluate, apply and disseminate new techniques by skilled health professionals and an informed public. Medical genetic services are rapidly extending but there is concern about rising demand, inadequate infrastructures and the ethical and social implications of the changes.Genetic screening at the population level is expending rapidly, before an appropriate evaluation. Not only should genetic screening do more good than harm at a population level, it would do well to make individual sense to the person being screened. The availability of genetic tests at low cost may lead to the systematic offer of screening tests without the appropriate medical environment for providing information prior to testing as well as comprehensively explaining the results afterwards.

E-PS11. The universality of the human issues raised by genetics

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The priority that governments can give to genetic services varies between countries, particularly between those in the developing and the developed world. This is determined by factors including overall resources available and competing priorities. In August 2001, the Department of Health of the Republic of South Africa (RSA) announced the launch of the 'Human Genetics Policy Guidelines for the Management and Prevention of Genetic Disorders, Birth Defects and Disabilities'. Alongside this, genetics training courses for nurses and midwives from the nine provinces took place with the support of the World Health Organisation. Three of the one-week courses, involving colleagues from eight of the provinces, were organised jointly by the Department of Health (RSA) and the Department of Clinical Genetics, St Mary's Hospital, Manchester (UK). The format and content of the training will be outlined. Both the resources available to and the cultural issues facing colleagues in RSA and the UK are different. However, examples will be presented to illustrate that the psychosocial impact of inherited disorders and birth defects are similar in the two countries. This suggests that while different priorities need to be set for genetic services in the developing and the developed world, the human issues raised for families and professionals are universal.

E-PS12. Twenty years of social work in clinical genetics in the Netherlands: Where do we stand and what do we need in the future

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From its beginning as a profession, social work has demonstrated a strong commitment to serving individuals and families whose health problems have influenced their psychosocial functioning in serious ways. In most clinical genetic centres in the Netherlands there are social workers involved in the genetic testing programs who provide psycho-education and support. Although the role of social workers in the psychosocial counselling for individuals and their families is widely accepted, there are only a few practice-based research reports.
In the Netherlands, the number of social workers in the clinical genetic centres has doubled in the last fifteen years. They offer individual support to counselees and their partners, they participate in formulating counselling protocols, and they develop models for professional help and psycho-education.
Recently it has become evident that a job description was needed to define the core competencies of the position for other (health) professionals, both in the field of clinical genetics and externally. The areas of results are well defined in the job description so it is now clear in which areas specific to genetics a social worker can offer help.
From a joint inventory of the activities carried out in recent years, we present an overview of the type of help most frequently requested and offered. Moreover, this inventory reveals which results from the psychological research in genetics have proved to be most useful in social work practice. It also reveals what issues we would like the research to focus on in the next ten years.

E-PS13. A randomized trial of three approaches to genetic counselling for late maternal age.

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Few outcome studies of genetic counselling for late maternal age have been reported, yet a significant proportion of pregnant women 35 and over are offered prenatal diagnosis; most will have genetic counselling prior to testing; these women must make complex decisions; and considerable resources are committed to this service. In our region, population 1.4 million, virtually all women are seen by a genetic counsellor before their first prenatal test. In 2000, 1149 women were counselled. Before 1999, counselling occurred in a one-on-one session. In the late '90s we developed a decision aid to facilitate communication of facts about prenatal diagnostic tests, focus patient views, and aid choice of testing. In a trial of that decision aid, knowledge significantly increased, decisional conflict significantly decreased, anxiety about testing did not change, and acceptability was reasonably high. In 2000, we initiated a randomized controlled trial to compare one-on-one counselling, group counselling of 4 women/couples, and use of the decision aid. All three approaches had the same content. We hypothesized that counselling in any form would improve knowledge, reduce decisional uncertainty and reduce levels of distress associated with prenatal testing. Secondly, we hypothesized that there would be no statistical or clinically significant difference between the three groups. Our results demonstrate that each approach has it's advantages: although people prefer one-on-one counselling, they learn best in a group or decision aid setting, and the least decisional conflict is found with the decision aid. Importantly, we find no clinically significant difference between the three approaches.

EMPAG Concurrent Sessions

E-C 1. Screening Issues

E-C01. Attitudes of Dutch general practitioners, pediatricians and gynecologists towards cystic fibrosis carrier screening

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Objective To investigate attitudes of general practitioners (GPs), pediatricians (PEDs) and gynecologists (GYs) towards cystic fibrosis (CF) carrier screening and to determine factors influencing these attitudes.
Methods A questionnaire developed by Hofman et al (Acad Med 1993; 68: 625-32) containing questions about knowledge and attitudes towards genetics (tests) was adapted to the Dutch health care system. Questionnaires were sent to randomly selected GPs (n=200), GYs (n=300) and PEDs (n=265). Multiple logistic regression

identified predictors of positive attitudes towards CF carrier screening.
Results The response rate of GPs, GYs and PEDs was 64%, 69% and 72%, respectively. Of the respondents 63% of GPs, 69% of GYs and 71% of PEDs agreed that couples should be tested if they ask for it. Of the respondents 32% of GPs, 40% of GYs and 32% of PEDs favored routine screening when a hypothetical error free inexpensive test would be available. However, after explaining limitations in the test sensitivity (95% detection rate for individuals) these percentages decreased to 16%, 19% and 25%, respectively. Predictors for offering routinely CF carrier screening in the latter situation were [OR (95%CI)]: considering the test sensitivity less important (GPs: [4.2 (1.4-12.3)]; GYs [6.2(1.9-20.7)]), high perceived risk of having a child with CF (GYs: [4.0 (1.2-13.9)]), providing genetic counseling (PEDs: [4.2 (1.2-15.0)]) and reassurance when both partners test negative (PEDs: [4.3 (1.6-11.8)]).
Conclusion Although approximately two-thirds of physicians support performing CF carrier screening when couples ask for it, more reservations are present among these physicians for routinely offering CF carrier screening.

E-C02. Feasibility and acceptability of two screening strategies for haemochromatosis, report of phase one of a randomised controlled trial.

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Haemochromatosis, a treatable, adult-onset condition of progressive iron overload is amenable to screening. Initial enthusiasm for screening to increase early diagnosis has been modified since the identification of the HFE gene. The risk of disease attributable to the at-risk genotypes, and disease progression in those diagnosed is not established. Screening trials will identify individuals in whom these questions can be evaluated. Evaluation of these programmes should include evaluation of sensitivity and specificity and all the components of the programme e.g. uptake, population characteristics and effectiveness of treatment.
Design: Randomised controlled trial of two screening strategies.
a) Biochemical screening for iron overload followed by genetic analysis and clinical assessment
b) Genetic screening for the at risk genotype followed by biochemical testing for iron overload and clinical assessment.
Sample: General practice population aged 30-70 stratified by age and sex
Findings: 1438 individuals were invited; initial acceptance was approximately 30%. Uptake was higher in females than males, specifically in older females. Middle-aged men who would be expected to have a higher risk of expressing the at-risk genotypes were less likely to accept the offer of screening.
The allele frequencies for the haemochromatosis associated alleles were greater than would be expected from previous population studies.
Discussion: In this pilot of population based screening in primary care, the low uptake of screening and the characteristics of the population that accepted screening have implications for the design of treatment or screening trials for haemochromatosis that merit further investigation.

E-C03. Coping strategies of pregnant women after "triple-diagnostic" and those of their partners

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Objective: To compare coping strategies of pregnant women after the triple-diagnostic and of their partners. To point out the importance of the genetic counselling. Background: The triple-diagnostic has become a standard prenatal screening-method and involves a combination of three serum parameters with personal data to define the individual's probability for a fetal trisomy 21. This screening often takes place without giving sufficient information. A "bad result' is mostly equated with a handicapped child. Methods: Our investigation was carried out by questionnaires. The answers of 92 women after "triple-diagnostic', genetic counselling and prenatal diagnosis and of 52 of their partners were evaluated. Results: Only 2/3 of the women were given information about this screening-method by their gynaecologist. A quarter of the partners had been informed by their wives or by the gynaecologist. Half of them had received this information only at genetic counselling. After a pathological triple test, 86% of the pregnant women and 90% of their partners felt depressed; 2% felt activated. After genetic counselling, there was a significant increase to the activated mood (women: 52% were depressed, 34% felt activated; partners 58% and 40%). The coping strategies showed the most significant variation in active coping: after risk information, women 17%, partners 10%, and after prenatal diagnosis, 55% and 45%. 25% of the pregnant women refused invasive prenatal diagnosis after genetic counselling. Conclusion: Genetic counselling plays an important role in the development of positive coping strategies. It should provide information and therapeutic support for pregnant women and for their partners.

E-C04. Serum screening uptake and attitudes towards Down's syndrome

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Prenatal screening for Down's syndrome is now offered in some form to most pregnant women in the UK. However, very little is known about the attitudes that women hold towards Down's syndrome and how such attitudes may influence screening choices. This paper presents findings from a study based in an antenatal clinic in the north of England where serum screening was offered to all women attending for antenatal care. Over a six-month period, women in the first trimester of pregnancy were asked to complete a questionnaire that incorporated measures of cognitive, emotional and experiential aspects of attitudes towards Down's syndrome. An objective measure of the participants' serum screening uptake was then collected at a later date from patient records. The findings suggest that regardless of attitude towards Down's syndrome most women accept screening tests. All of the women with the most unfavourable attitudes towards Down's syndrome accepted screening as did 67% of those with the most favourable attitudes - despite the latter group holding unfavourable views towards termination for the condition. The findings have implications for the issues of informed choice and the perceived 'routineness' of prenatal screening tests.

E-C 2. Cultural and Ethical Issues

E-C05. Thalassaemia carrier testing in pregnant Pakistani women: perceptions of 'information' and 'consent'.

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Introduction: The literature on genetic testing suggests that one of the main objectives of screening programmes is to allow individuals to make informed decisions. However, there is no published research on whether women value informed consent for antenatal thalassaemia carrier testing.
Aims: To ascertain pregnant Pakistani women's perceived value of 'informed consent' for antenatal thalassaemia carrier testing and their perceived pre-test information needs.
Methods: In study 1, 110 Pakistani women tested and not found to be thalassaemia carriers completed a questionnaire, 14 of whom were also interviewed. In study 2, 36 women identified as carriers/possible carriers completed a questionnaire and were interviewed. The questionnaires assessed women's knowledge and understanding of antenatal tests; their attitudes toward antenatal care and tests for fetal abnormality; their knowledge and understandings of thalassaemia; and their pre-test information preferences. The interviews explored these domains in more depth and investigated women's beliefs about 'informed consent'.
Findings: Women had received little or no pre-test information and said that they would have preferred to be informed that they were being tested for thalassaemia carrier status, but they did not expect, or express a desire, to be asked for their 'informed consent' for antenatal thalassaemia carrier testing.
Discussion: While women wanted pre-test information in order to be 'informed' of antenatal thalassaemia carrier testing, they were less concerned about being asked for their 'consent'. This finding is discussed in the context of the way in which service delivery is organised.

E-C06. Breast cancer: South Asian patient's experience, attitudes, beliefs and perception of risk.

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Background: Incidence of breast cancer among South Asian women living in the UK is increasing. We know little about how these women make sense of the condition or how health care services can best support this patient group.

Aim: The study aims to examine health care pathways, attitudes, beliefs and genetic perception of risk factors in South Asian women with breast cancer compared with 'white' women.

Method: The study comprised of two groups of women attending breast clinic because of a diagnosis of breast cancer. The study comprised 25 South Asian and 15 'white' women' diagnosed with breast cancer, matched for diagnosis, age, and socio-economic class. This enabled us to explore the role of ethnicity in mediating women's responses to the illness. For further context, our work also included the experience of the women's main family carer (n=29) and a health professional involved in their care (n=15).

We collected information using semi-structured qualitative interviews, which were then subject to detailed content analysis.

Findings: The main themes emerging from the analysis include access to service delivery, knowledge of breast cancer, health beliefs, coping strategies, cultural beliefs regarding genetic and other causes of breast cancer, communication about the condition within the family, and the role of health professionals in offering support. The presentation will conclude by exploring how culturally sensitive provision would improve the care of women with breast cancer.

E-C07. Genetic Testing for Hearing Impairment- Different Motivations for the Same Outcome

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The recent discoveries of genes involved in hearing impairment opens new options for families and individuals with hearing impairment, but also raises new ethical dilemmas. Our study aimed at evaluating the intensions, as well as the reasons, of Israeli Jewish parents of hearing impaired children to opt for or against genetic testing and prenatal diagnosis for deafness. Questionnaires were filled by 139 parents, showing a very high interest (87%) in genetic testing, and lower interest (49%) in prenatal diagnosis. Althogh part of the Jewish population in Israel do not comply with genetic services, due to religious restrictions, the high interest in testing was found across all religious sectors (secular, traditional, orthodox and ultra-orthodox); however, some of the reasons for undertaking such a test were very different between the sectors. We conclude that genetic testing would be welcomed by parents from a wide range of communities, including those which usually do not apply for genetic counseling and testing, if it is offered in accordance with their cultural norms and beliefs.

E-C08. Uses of the body and informed consent for participants in predictive medicine research

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On the occasion of the creation of a DNA bank for research in predictive medicine in the south of France, this sociological study deals with an ethical and juridical norm and its application: the principle of free and informed consent of DNA donors-participants. This consent is often said to favour the expression of the patients' "autonomy" towards "medical power": it would make sure that individual freedom in the uses of the body is put in confrontation with the biomedical enterprise. Previous studies have addressed the question of the quality of the consent given by participants. This study rather addresses the way in which they "frame" the consent (they give a meaning and their position in relation to this meaning). Not only their position regarding the procedure but also that towards the concept of consent itself are investigated. The access to this framing is achieved through thorough interviews (planed number = 50). This speech on consent is then mirrored with the participants' uses of the body (or elements issued from the body): these uses are apprehended here through the meaning given by the DNA donors to their involvement in research. The first results show different typologies of the body usage: the body submitted to medical activity, the body medically auto-supervised and "property" of the individual or also the body subjected to collective interests. I will try to show that these modalities of use involve different ways of understanding and of using the principle of informed consent.

E-C09. Am I My Brother's Keeper?: Outlining Rights and Responsibilities in the Context of the Human Genome Diversity Project

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There are many obstacles to continued scientific research, the Human Genome Diversity Project (HGDP) specifically. The main obstacles appear to be differing philosophies toward human rights and responsibilities. On the one hand, Western researchers impute ownership rights upon human body parts. This view is repugnant to the culture and beliefs of many indigenous people. However, indigenous peoples and some Western legal scholars maintain that a duty exists for individuals to further the survival of others through scientific research. Still, this duty conflicts with a duty that may exist to future generations not to tinker with the common heritage. The issue of human genetic diversity presents an interesting paradox: genes are responsible for one's uniqueness, and simultaneously, genes are an omnipotent component of every human being. Achieving a balance between preserving the integrity of our genetic heritage and granting ownership rights in furtherance of scientific knowledge is a perplexing challenge. Yet, at least one group of indigenous peoples has achieved such a balance, and they justified their agreement by articulating a duty to others to help understand and cure a particular disease. Perhaps if we all recognize that we are our brother's keeper, we can work together to further the aims of science while at the same time maintaining respect for proud cultures.

E-C 3. Psychological Support, Family Issues and Impact of Genetic Disease

E-C10. A Pilot Project on Rehabilitation in Huntington's Disease: Three Years Experience in Italy.

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Huntington Disease (HD) is a genetic, chronic, neurodegenerative disorder for which there is no known cure. In the past, rehabilitation in HD was justified on humanistic compassionate ground but now it has a more scientific fundament and theoretical support in recent studies using animal models that show beneficial effects of environmental stimulation on disease progression and suggest a possible positive impact of environmental factors on individuals affected by HD.

A pilot research project on the evaluation of rehabilitation effects in HD has been started in Italy in 1999. Fifty patients with a clinically and genetically confirmed diagnosis of HD have been enrolled in a rehabilitation protocol including neuromotor and cognitive therapy. Patients' conditions were assessed at patients' admission and discharge. Further additional data were collected from patient's and families' interviews. First results are encouraging because we can remark notable improvements both on motor control and psychological conditions and we have also found positive effects on family relationships and on caregivers well being state.

Analyses of data are currently performed.

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E-C11. Communication with relatives about predictive genetic testing for cancer predisposition

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Individuals who have a genetic test for breast/ovarian cancer

predisposition are required to pass on information to their relatives. The purpose of this study is to explore communication in the family about cancer and predictive genetic testing for breast and ovarian cancer predisposition amongst women at increased risk of developing breast and/or ovarian cancer due to their family history. 15 women attending the RMH Genetics Clinic were recruited into the study. They were interviewed at least one week prior to receiving their BRCA1/2 test result and again 6 months following the test result. A grounded theory approach was adopted to analyse the interview transcripts. The findings indicated that cancer or deaths had affected family relationships, with some relationships being strengthened and others becoming distant. Many women had talked to other family members about testing. Women anticipated being selective in which family members they would tell about their test result. Several women experienced difficulty in talking to relatives about their test result. The findings illustrate that the nature of relationships and differing opinions within the family regarding genetic testing are likely to have an impact upon dissemination of information about predictive testing to relatives.

E-C12. Myotonic Dystrophy and the marital relationship

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Background: Myotonic Dystrophy (MD) is a multi-system disease with symptoms including muscle weakness, especially in facial muscles and distal extremities, cardiac and gastro-intestinal problems. Cerebral symptoms of MD include (daytime) sleepiness, loss of energy, mental slowness and lack of initiative. Some studies suggest a change of personality. Based on clinical findings 3 phenotypes have been identified: mild, classical, and congenital. MD is inherited in an autosomal dominant manner. Offspring of an affected individual have a 50% chance of inheriting the causal mutation.
These symptoms not only affect the patients' life but also that of their spouses. Couples seem to respond to this variety of symptoms in different ways. Some apparently cope with these problems and go on living their (more or less adjusted) lives. Others express having trouble in doing so and keep experiencing this trouble for a prolonged period of time.
Objectives:
1. How does MD influence the lives and relationships of patients?
2. Which factors contribute to people's ability to overcome the problems caused by MD?
Methods: 100 patients of the outpatient clinic and their partners are interviewed about their relationship and the way they feel it has been affected by MD. Information on present symptoms of MD, personality, coping style, family functioning, anxiety and depression is obtained through questionnaires. While subjects watch funny pictures, facial expressiveness is assessed by experienced judges.
Results: Until January, 57 patients (58% males) and their partners (40% males) have enrolled. Mean age 46,5 years (sd = 8,8).

E-C13. Putting HD into words: exploring the virtual narratives of the Italian HD support group online.

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Three years ago the Italian Association for Huntington's Disease (A.I.C.H.-ROME) has developed a web site. It is a non commercial service and is the result of patients'and caregivers' voluntary work. In Italy it is the unique "interactive" website for HD.
Beyond any expectation, this virtual community interested to/involved with HD for many different reasons has been growing very rapidly: from 6 contacts a week, when the site was started, up to 1600 contacts in one month (November 2001).
Many messages have arrived: some messages are brief and simply ask for information, other messages are rather long and are aimed at sharing the intimate, emotional aspects of the individual's experience of the disease or at confronting with others about main life decisions such as to be tested or not, having or not children, having or not prenatal diagnosis, informing or not children about the disease in the family and so on.
These exchanges on line offer a unique opportunity of a glimpse to the heart of a support group for a severe, incurable, hereditary disease. A text analysis and a qualitative examination of the messages are currently performed. Our first inquiries are aimed at: providing a description of this virtual community; analysing the priorities given by the contributors to the different issues; exploring the way they communicate their ideas and emotions about the disease.

E-C14. Adult Survivors of child sex abuse in the genetic counselling consultation

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Given the prevalence of sexual abuse in our society, with a large proportion of the abuse taking place within families, it is clear that any health professional will be dealing with clients who are survivors of childhood sex abuse.
This paper explores how a pre history of childhood sexual abuse may impact the genetic counselling consultation. The genetic counselling situation is possibly the only one in which taking a family tree is such a large part of the consultation. It can expose people to thinking about their relationships within the family and bring forward painful memories. A faulty gene running through the family can highlight the legacy of abuse or the 'inheritance' of the problems associated or passed on with it.
Implications for genetic counsellors discussed in this paper are:
Disclosure of sexual abuse.
The specific genetic implications of incest.
Non disclosure of genetic information to family members because of a history of abuse.
Occasional cases of 'munchausens syndrome' particularly where prophylactic surgery may be considered have been reported.Could some of these women have been sexually abused?
One of the predictors of abnormal grief reaction following termination of pregnancy is incest and sexual abuse. As genetic counsellors we are often supporting women in this situation.

E-C 4. Genetic Counselling for Hereditary Cancers

E-C15. Women at increased risk for breast cancer (BC) attending a regular surveillance program. Preliminary results of the baseline measurement.

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Background: The MRISC study is a surveillance program for women at risk of BC due to a genetic predisposition or family history. Surveillance consists of bi-annually physical examination and yearly mammography and MRI-scan. The estimated actual risk of developing BC in terms of a cumulative life time risk (CLTR) is trichotomiseda. Category 1 implies a CLTR of 60-85%, 2: 30-50%, and 3: 15-30%.
Objectives: Identify (a) the relation between CLTR, coping strategies and psychological distress; (b) the relation between risk of developing BC as perceived by the participant (adjusted for CLTR), coping strategies and psychological distress.
Methods: Participants completed a questionnaire 2 months prior to their surveillance appointment, containing: the Hospital Anxiety and Depression Scale, the Impact of Event Scale, the Psychological Consequences Questionnaire and the Utrecht Coping List. The participants perceived risk was measured in terms of cognition and affect. Multiple linear regression and polychotomous ordered logistic regression analysis were used to identify the relationships between actual and perceived risk on the one hand and psychological factors on the other.
Results: 241 women answered their baseline questionnaire (mean age 40 years). 11% belong to category 1, 53% to category 2 and 36% to category 3.
CLTR is positively related to avoidance and negative psychological consequences ($p < 0.05$). Perceived risk is positively related to a depressive coping style ($p < 0.02$).
Conclusion: Having a higher CLTR means displaying more avoidance and experiencing more negative psychological consequences. Women perceiving their risk of developing BC as high display a depressive coping style.

E-C16. The meaning of risk: Women's perceptions of the genetic risk of breast and ovarian cancer following BRCA1/2 mutation searching

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The literature suggests that there is a divergence between the ways in which individuals with a family history of breast/ovarian cancer perceive their risks of developing cancer and the risk estimates provided by clinicians. However, there has been little research that explores the meaning of risks for individuals who are deemed at risk because of their family history, and none that has looked at how women who have previously been affected with cancer make sense of their (potentially) increased risks following DNA-testing.
This retrospective study of affected women who had undergone BRCA1/2 mutation searching investigated their perceptions of developing cancer. In-depth interviews were undertaken with 30 women. These explored their risk perception and the impact of genetic testing on their risk perception.
The data suggest that following their initial diagnosisall women were very aware of their recurrence risk. However, most reported that their anxiety about developing cancer had decreased over time. For some the probability of developing cancer was perceived as reducing over time, whilst others described their risk of cancer as constant but their ability to accommodate risk within their lives changed. Women's understanding of their (potential) inherited risk of cancer involved reconciling their previous experiences with their expectations of the future. Thus, some women, did not regard genetic risk as a threat, whilst others reported an increase in anxiety on learning their risks of developing a second primary cancer. The implications of these findings for theoretical accounts of risk perception will be discussed.

E-C17. Women from HBOC families during the BRCA genetic testing process : lay and providers interactions

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The objective of this study was to describe medical and social interactions about BRCA genetic testing and how helpful they were perceived by the patients during the cancer genetic testing process.A prospective cohort study is ongoing including all women who attended at one French cancer genetic centre after a first biological sample was analysed for mutation identification (BRCA1/2). Closed questionnaires were administered before and after the occurrence of the 2nd cancer genetic consultation. This consultation aims to confirm the decision to be tested. No biological results were given during this consultation.Preliminary results were analysed (N=82 ; mean age 46, SD=11; 70% affected by cancer). The medical providers consulted about the decision to be tested were: the Cancer Geneticist (82%), the Gynaeco-Obstetrician (44%), the General Practitioner (42%) and the Clinical Psychologist (7%). When they occurred, these encounters were considered helpful by 92%, 75%, 67% and 62% for Cancer Geneticist, Clinical Psychologist, Gynaeco-Obstetrician and General Practitioner respectively. In the family, spouses and sisters were consulted in 40%, and mothers in 26%. These encounters were considered helpful in 76%, 60%, and 44% for sisters, mothers and spouses respectively. All the sources consulted were in their overwhelming majority favourable to genetic testing. An analysis of medical and psychological factors related to information exchange and information seeking will be investigated further.These results highlight the need for education in cancer genetics, not only for primary care providers but also for the social network of those concerned by genetic testing.

E-C18. Theory of Engagement: A model for predictive test counselling

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The theory of engagement emerged from grounded theory work with HNPCC families having predictive genetic testing (McAllister, 1999; 2001), and differs from existing general psychological theories because it is grounded in the experiences of high risk families, and makes specific predictions about adjustment to the results of predictive genetic testing. According to the theory, it is not test result per se, but prior engagement status in combination with test result that predicts post-test adjustment. Intense engagement prior to predictive testing is associated with feelings of relief, and often satisfaction with the discovery of mutation carrier status in HNPCC families. These mutation carriers may feel that they are better off than members of the general population because their carrier status is perceived as a gateway to care. These feelings are not always shared with their less engaged relatives. A proposed model for pre-test counselling for cancer predisposition is presented that may enable more appropriate targeting of clinical resources, cost-effective practice, and the likelihood of decreased patient distress and increased patient satisfaction. It may also prevent future difficulties in adjusting to predictive test results.

E-C19. Impact of predictive genetic testing for hereditary non-polyposis colorectal cancer (HNPCC)

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Background
This longitudinal multi-centre Australian study examined the response of people at high risk for colorectal cancer to predictive genetic testing for hereditary non-polyposis colorectal cancer (HNPCC). There were four assessment times of participants: prior to, and 2 weeks, 4 months and 12 months after notification of test result.
Results
104 people have completed the baseline questionnaire, of whom 56 are female and 45 male. Almost all have received genetic test results and also completed the second questionnaire, 88 completed the third and 76 the fourth.
Measures of anxiety, depression, and intrusive and avoidant thoughts about being at risk for colon cancer (as measured by the Impact of Events Scale [IES]) were included in the questionnaires. Results show marked differences between carriers and non-carriers two weeks post notification, particularly for the IES measure. The difference between these two groups for IES scores remained statistically significant at 4 and 12 months post testing, having adjusted for age and baseline levels of IES. However, all measures decreased between time 2 (two weeks after the result was given) and 12 months for both groups.
Most non-carriers appear reassured and did not have colorectal cancer screening in the first year post genetic testing: 12% of non-carriers have had a colonoscopy, compared with 58% of carriers.
Conclusion
At this stage, there is no evidence that having a predictive test for HNPCC is causing psychological distress and compliance with advice related to screening is being observed.

E-C20. The influence of consultants' communication and information-giving behaviours on patient outcomes: A multi centre study of genetic counselling with women from high risk breast cancer families

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This longitudinal study aimed to document i) the information-giving and patient-communication styles of clinical geneticists and genetic counsellors (consultants) in familial breast cancer clinics, and ii) assess the impact of these behaviours on women's knowledge, whether their expectations were met, satisfaction, risk perception and psychological status.
158 women from high-risk breast cancer families completed self-report questionnaires at two weeks pre-and four weeks post-consultation. The consultations were audiotaped, transcribed verbatim and coded.
Multivariate logistic regressions showed that women who had prophylactic mastectomy (p=0.00) and oophorectomy (p=0.01) discussed had significantly more expectations met and a greater reduction in breast-cancer-related avoidant thoughts (p=0.07 and p=0.09 respectively). Women who received a summary letter of the consultation and reported reading it, experienced significantly lower generalised anxiety (p=0.01), lower breast cancer specific anxiety (p=0.08) and were significantly more likely to be accurate in reporting their perceived risk (p=0.02). Women whose consultant used more supportive behaviours experienced significantly more breast-cancer-related intrusive thoughts at the four weeks follow-up (p<0.001). Women who received more supportive behaviours were not significantly more anxious before to genetic counselling.
Discussing prophylactic surgery led to better psycho-social outcomes. Providing women with a letter summarising the consultation reduced anxiety, increased accuracy of risk perception and was a useful adjunct to the consultation. Greater use of supportive and counselling behaviours appeared to increase intrusive thoughts, at least in the short term. Longer follow-up may have shown a reduction in emotional response. Identifying methods to assist consultants to effectively address emotional issues may be helpful.

E-C 5. Professionals' and Family Attitudes towards Genetic Testing

E-C21. Attitudes of persons at risk for late-onset neurodegenerative disorders and for hereditary cancer diseases towards molecular genetic predictive diagnosis

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Objective: To compare the attitudes of persons at risk (RP) towards genetic predictive diagnosis (PDD).
Background: Huntington's disease (HD) and heredoataxias (HA) are late-onset neurodegenerative disorders with (HD) and without (HA) dementia. Both are incurable. Hereditary cancer diseases (HC) can be prevented by prophylactic methods. DNA analysis is available.
Methods: Our investigation was carried out by questionnaires. Answers from 300 RP for HD, 30 for HA and 55 for HC were evaluated.
Results: About 55% of RP for HD, 73% for HA and 85% for HC wish to undergo PDD. The main reasons are to obtain certainty about risk status (HD: 78%), to plan for the future (HA: 68%) and to obtain prophylactic therapy (HC: 80%). 33% of the RP for HD, 13% for HA and only 4% for HC refuse PDD. The main reasons against PDD are psychological problems (HD: 79%; HA, HC: 50%). 2/3 of the RP for HD, 1/3 for HA and only 1/5 for HC think that there are problems in taking PDD generally. Most problems are seen in coping with the result of PDD (psychological problems; HD: 77%; HA: 60%; HC: 45%), with insurance companies (40%, 20%, 55%) and with social surroundings (36%, 20%, 18%). Psychotherapy would be accepted by 93% (HD), 83% (HA) and only by 25% of RP for HC.
Conclusions: There are different attitudes towards PDD in the three groups because of the different symptoms and the possibilities of preventing in the case of cancer. Genetic counselling has to consider these features.

E-C22. Progress in genetic : the opinion of the deaf patient and their families ?

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Deafness is the most frequent sensorial defect. One in a thousand children present with profound deafness at birth. As this handicap affects communication, deafness impedes language acquisition, speech development and social integration. The particularity of this handicap is the existence of a deaf community with their own language and culture. The recent progress in the identification of the genes responsible for isolated deafness have open new technical possibilities and pose significant ethical problems. We send questionnaires to hearing parents of deaf children and to deaf adults. The aim of these questionnaires is to discover if patients know the etiology of their deafness, if they want to know it, if they have access to a genetic counselling consultation and if not why, what are their thoughts about the possibility to pratice antenatal diagnosis, and therapeutic abortion. We analyse more than 200 responses. We try to determine if the reponses are influenced by the audiologic status of the responder, the severity of the defect, its association with an other handicap or others parameters.

E-C23. Relevant issues in genetic counseling for familial dementia: a study on attitudes towards testing in at-risk relatives.

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Some familial forms of dementia were found to be transmitted as mendelian traits, such as early onset Alzheimer disease and frontotemporal dementia with parkinsonism, which were associated with mutations in the PS-1 and MAPT genes, respectively. In these cases, the mutation is characterised by autosomal dominant inheritance, with high penetrance. Thus, a test with high predictive value can be offered to the at-risk healthy relatives of patients.
The present study is aimed at: i) evaluating the attitudes towards genetic testing in relatives of patients with familial dementia; ii) developing a specific protocol for genetic counselling, including presymptomatic testing, for families with mendelian forms of dementia.
Thirty-five relatives of patients with familial dementia were recruited. Participants were tested by an extensive psychological assessment including several rating scales; the attitudes towards genetic test were evaluated by using a specific tool (Roberts, 2000). About 70% of participants expressed probable intentions to seek for the test. Participants reported test benefits as more important than limitations and risks (p=.0019). Staying on top of future treatment, as well as planning the future, were considered the most important items in favour of the test. Psychological and sociodemographic characteristics do not seem to influence attitudes and intentions towards genetic testing. Two families in which a MAPT gene mutation segregates with frontotemporal dementia were identified; some at-risk relatives requested to be enrolled for the genetic testing procedure. On this purpose, a multidisciplinary team is developing a pilot protocol for genetic counselling. The preliminary experience will be reported.

E-C24. Awareness of the contribution of genetic factors to aetiology amongst individuals with bipolar disorder.

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Bipolar disorder is a chronic, mental health condition with a multifactorial, polygenic aetiology. The illness is characterised by episodes of both mania and major depression, typically occurring in cycles.
The aim of this exploratory study was to assess knowledge of the possible causes of bipolar disorder and to discover the level of concern amongst participants that other family members may be at risk of developing the illness. 22 adults diagnosed with bipolar disorder were interviewed, using a semi-structured interview schedule. Data were analysed to see if there was an emerging difference between those with a family history of mental illness and

those individuals who were isolated cases.
The results showed that 15/22 (68%) knew that family history plays a role, with 13 then explicitly stating genetics as a cause. 77% cited stressful or traumatic life-events as a cause of their illness. More than half the sample overestimated both the population lifetime risk of bipolar disorder and the lifetime risk for those with an affected parent. There was no evidence that those with a family history overestimated the risks compared to isolated cases. Those who overestimated population risk were also overestimating family risk for developing bipolar illness. Almost half of the sample (10/22) had experienced some degree of worry about other family members becoming manic-depressive.
These findings suggest that genetic counselling and information may be useful to this population.

E-C25. General Practitioners And Predictive Genetic Testing For Late Onset Diseases: Their Opinions And Their Perceived Role

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A sample of 356 GPs received mail questionnaires in combination with telephone prenotifications and reminders to assess their opinions (1) on the sense of predictive testing for breast cancer, thyroid cancer, Alzheimer disease and Huntington's disease, (2) on the acceptability of testing a child at the parents' request and an adolescent at his/her own request, (3) on their own role in the context of predictive testing. Multiple-choice and open-ended questions were used. Sixty percent returned the mail questionnaire. One third of them had at least one patient who had asked for information about a predictive test and/or who had such a test performed. The following pattern in the GPs' judgments about the sense of predictive testing was revealed: the test for thyroid cancer was considered as more sensible than the test for breast cancer, both were more sensible than the test for Huntington's disease and all three were more sensible than the test for Alzheimer disease. Predictive testing for Huntington disease for an adolescent at his/her own request was judged as more acceptable than for a child at the parents' request. The GPs' explanations for their judgements will be presented. Six percent were convinced that GPs have no role at all to play in the context of predictive testing for late onset diseases. Regarding the type of task, most GPs focussed on gate-keeping aspects, going from the provision of information, over making referrals, to being more directive. The research was funded by the Flemish Interuniversity Institute of Biotechnology (VIB).

E-C 6. Prenatal Testing: Decision Making and Outcomes

E-C26. Informed choice to undergo prenatal screening: a comparison of two hospitals conducting testing either as part of a routine visit or requiring a separate visit

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Background:
The wide variation in uptake of antenatal Down syndrome screening is associated with the method of conducting screening. Uptake is lower when screening is conducted at a test-specific separate visit compared with a routine visit. It is not known if informed choice also varies with the method of conducting screening. Women may find it easier to decline screening if it is conducted at a test-specific separate visit. Conversely women may find it easier to accept screening if it is conducted as part of a routine visit.
Aim: To compare rates of informed choice for Down syndrome screening where it is conducted at a test-specific separate visit or as part of a routine visit.
Participants: 1499 pregnant women offered Down syndrome screening.
Setting: Two hospitals in the UK offering the same Down syndrome screening test.
Outcome measure: the multi-dimensional measure of informed choice, comprising three core constructs: knowledge about the screening test, attitudes towards undergoing the screening test and screening uptake.
Results.
Rates of informed choice to decline screening were the same at both hospitals (23%). Rates of informed choice to accept were higher at the hospital conducting tests as part of a routine visit than as a separate visit (41% vs 21%).
Conclusion: Screening conducted as part of a routine visit is associated with higher rates of informed choice, than if it is conducted at a test-specific separate visit. An experimental test of this observation is underway.

E-C27. Pregnancy Outcome After Genetic Counselling For Prenatal Diagnosis Of Chromosomal Anomaly With Low Risk Of Severe Clinical Significance

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The aim of the study was to evaluate the psyco-social impact of the identification of a foetal chromosomal anomaly on couples who underwent prenatal diagnosis in the years 1996-2000 and were referred for genetic counselling to our service.
Inclusion criteria were the identification of a chromosomal anomaly that may have no phenotypic consequences or only mild ones: variant, mosaicism (I-II level), balanced familial inversion or translocation, familial or denovo extra structurally abnormal chromosomes (ESACs) and sex chromosomes aneuploidies.
Exclusion criteria were: presence of a malformation detected by ultrasound, or autosomal aneuploidy, or unbalanced chromosomal anomaly.
Both parents have been interviewed and asked to fill in a questionnaire, consisting in two parts (general information, scale for the assesment of anxiety).
RESULTS
A total of 36 couples were included in the study and 30 agreed to participate: 13 sex-chromosome anomaly, 11 mosaiscisms, 4 translocations, 1 ESACs, 1 variant. Only 10/30 had a formal counselling by the obstetrician before the prenatal diagnosis.
2 couples (1 mosaicism II level, 1 45,X/46,XX) underwent an induced abortion, 16 of the 28 remaining couples who decided to continue the pregnancy, did it after the genetic counselling. There was no differences in the level of anxiety between the 12 couples who have decided to continue the pregnancy before the genetic counselling and the others 16.
CONCLUSION
Couples undergoing prenatal karyotiping should be counselled before performing the tests.
The genetic counselling has an important role in informing parents and in making the decision to continue the pregnancy.

E-C28. Defining a psychological intervention program for women undergoing interruption of pregnancy after prenatal diagnosis.

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Interruption of a pregnancy (TOP) after prenatal diagnosis is a challenging issue on genetic counselling. The need of psychological intervention to facilitate the adjustment process is well described in literature; however, few specific empirical data on the decision process and coping with loss of a wanted pregnancy are available. Our work focuses in those issues using two different theoretical approaches: the Ottawa decision support framework and coping with critical life-events, including cognitive-narrative perspectives on loss. Our main objectives are: a) to define the weight of difficult adjustment factors: clinical and social data, coping responses, decision-making determinants and woman's metaphors for the event; b) to check which coping strategies are effective preventing anxiety and depression; and c) to define a program of psychological intervention. Our protocol includes 4 sessions, with evaluation at 15 days and 6 months after TOP. At the first post-TOP evaluation, a structured interview is used, covering social-demographic data, decision determinants (perception of the decision, perception of significant others, resources available for the decision-making process) and specific loss issues (social support, knowledge about the process, grief thoughts and metaphor for the episode), Moos Coping

Responses Inventory, Beck Depression Inventory (BDI), Zung Anxiety Scale (SAS) and Generic Decisional Conflict Scale. At the 6th month evaluation, a structured interview, with BDI and SAS, is used.
We discuss the guidelines for the assessment and intervention on this critical life-event, focusing data on the importance of decision support, the coping process, emotional and cognitive intentional attribution of the experience and its metaphor.

E-C29. Feticide and late termination of pregnancy: impact on parents and health professionals

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In some countries, legislation permits termination of pregnancy after the diagnosis of fetal abnormality at gestations beyond fetal viability. Prior to such a late termination, it is necessary to ensure that the baby is dead prior to the induction of labour. In the United Kingdom this procedure, feticide, is usually carried out in specialist fetal medicine units. There are no published data concerning how parents react to this procedure nor how it impacts on those health professionals carrying it out.
This paper will use questionnaire and interview data collected from twenty-eight women and their partners, whose pregnancies were terminated with the use of feticide after the late diagnosis of a fetal abnormality. We will describe the impact of this procedure for both men and women, compared with parents undergoing termination for abnormality at earlier gestations without the use of feticide. Attitudes to and experiences of the procedure varied among parents. Overall, no relationship was found between undergoing feticide or not and maternal emotional well being although a year after a termination, there was evidence of higher paternal grief in those men whose partner had undergone a feticide.
We will also describe the findings from interviews with health professionals (both doctors and midwives) in fetal medicine units, which explored: their attitudes to the legal framework for abortion in the UK; the policies and practices that have evolved within the legal framework and Royal College guidelines; and the impact of this procedure on them as practitioners.

E-C30. Profiles and motives of couples choosing PGD

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Introduction.
The Academic Hospital in Maastricht is the only centre in the Netherlands that provides Pre-implantation Genetic Diagnosis (PGD). Between 80-100 couples are referred each year. This gave us the opportunity to investigate the characteristics and motives of the couples seeking PGD counseling.
The main question was:
Which couples opt for PGD and which factors might influence the course of treatment?
Method.
As part of the intake a Clinical Geneticist provides information about PGD. After that a Psychologist interviews the couple. A descriptive analysis of the first 50 interviews has been performed.
Results.
The presentation mainly deals with the first part of the question i.e. which couples opt for PGD. Roughly 40% of the genetic disorders that lead to the wish for PGD are autosomal dominant late onset disorders, another 45% are X-linked or autosomal recessive disorders and 15% of the cases have frequent early abortions due to translocations. Couples generally underestimate the impact of the disorder on quality of life. Women and men mention similar motives for parenthood. Couples feel medium to high time pressure in 60% of the cases. Alternative options to get a healthy child are considered in 50% of the cases but 70% express a strong preference for PGD. Sufficient social support is reported by 80% of the couples and only 10% does not want to discuss their problems with others. After the initial interview, 60% wants to continue, 10% declines to go on and 30% wants to reconsider their options.

EMPAG Poster Session

E-P01. Psychological adjustment of PKU children and the family

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Introduction: Phenylketonuria (PKU) is an inherited genetic metabolic disorder in which the enzyme required to digest phenylanine (Phe) is missing. If untreated, individuals with PKU develop high levels of Phe in their blood which can affect brain development and function. That's why low protein diet must be introduced from the birth. Nevertheless, several studies show higher incidence of behavioral problems, especially internalizing, in early-treated PKU children and suggest psychological perspective explaining them. Parental maladjustment to child's chronic illness and everyday stress managment related to the burden of special diet can be reasons for psychological problems of PKU children.
Aim: To evaluate the psychological adjustment of PKU children (as compared to healthy controls) and analyze it in the context of psychological impact of PKU on the family.
Methods: Parents of 37 early-treated PKU children (age 4-14 years old) and of 37 matched controls were asked to fill the Child Behavior Checklist (CBCL, Achenbach, 1991) and questionnaire on stress coping strategies (Elklit, 1996). Parents of PKU children answered the questionnaire on reactions to child's disease and it's impact on the family.
Results: PKU children have significantly more behavioral problems than healthy controls. They are more withdrawn, anxious/depressive, have more social and attention problems. The higher rates of internalizing and overall problems are related to parental maladjustment (feelings of guilty and anger) together with maladaptive (emotion-oriented) stress coping strategies. Two latter factors further indulging the child, that also predicts the psychological problems of PKU children.

E-P02. Are experiences with HD related to the attachment representation in adults at 50% risk for HD? An empirical exploration

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Introduction: Huntington's disease (HD) is a family disease. We introduced the attachment theory as framework to study the effect of childhood experiences with HD on adult functioning. According to the attachment theory a child forms on the base of daily interactions with his parents a mental representation of the relationship with his parents. A child is securely attached when he approaches his parents, especially when being frightened, tired or ill. He can also be attached to his parents in a dismissing (insecure-avoidant) or preoccupied (insecure-resistant) way. Once formed attachment representations are relatively resistant to change. The Adult Attachment Interview (AAI)[1] allows assessment at adult age of attachment representation, unresolved trauma and loss. We studied the attachment representation in adults at 50% risk for HD and explored its relation to HD experiences and family characteristics.
Method: 30 adults at 50% risk, having had a parent with HD in childhood, were administered the AAI. They completed a questionnaire concerning family characteristics and HD experiences. The differential qualities of the attachment categories were explored by multivariate modeling of HD experiences and family characteristics (i.e. optimal scaling techniques).
Results: 11 subjects were securely attached, whereas 14 were preoccupied attached and 5 dismissing attached; 16 out of them were unresolved regarding trauma and/or loss. Compared to non-clinical samples[2], fearful preoccupation and unresolved trauma were overrepresented. The relation between attachment representation and HD are currently being analysed. It will be discussed how the attachment representation is related to HD experiences and family characteristics.

E-P03. The situation and attitudes of patients suffering from a hereditary disease and those of their partners

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DNA analyses can help to differentiate diseases and to make a diagnosis certain. However, the psycho-social problems may increase with new diagnostics. One aim of our study was to investigate the attitudes of patients suffering from a hereditary disease and those of their partners. We wanted to investigate whether there are special features in coping with a heredity disease. Method: The study was carried out by questionnaires. Results: 68% of the patients and 27% of the partners had to restrict their professional life. About 20% in both groups had conflicts with family members. 52% of the patients considered that the difficulties of their situation had strengthened the partnership, whereas about 25% reported conflicts with their partner and, in 7%, the partnership was dissolved. For more than 70% of the patients, their partner is the most important person to help them to cope with the disease. 65% of the patients and 71% of the partners wished to undergo prenatal DNA diagnosis. More than 50% of both groups thought that the termination of pregnancy after a positive prenatal diagnosis is justified. Almost all patients confirmed that the doctor is important not only for giving information, but also for discussions regarding coping with the disease. Conclusion: Good family and social integration and close-meshed consultations by specialists form the basis for coping with a hereditary disease. Genetic counselling can provide the frame for genetic analysis. The partner has to be integrated into the counselling process and should be paid the same amount of attention.

E-P04. Cri du chat syndrome: qualitative analysis of behavioural phenotype

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Haploinsufficiency of 5p15.2-15.3 results in Cri du chat syndrome (CDC, OMIM 123450), which is a well-recognizable genetic condition with characteristic facial dysmorphism, organs anomalies, psychomotor and mental retardation. In contrast to routinely published convictions of severe mental and behavioural incapacity of children with CDC, more specific data on their abilities and educational needs are not available. The main aim of our work was a detailed qualitative study of the behavioural profile of CDC, starting with a 6-year-old boy with characteristic clinical features confirmed by cytogenetic findings. Our method is based on the analysis of single picture observations taken from video documentation during child directed therapeutic playwork in a Montessori structured environment. The synthesis of our observations revealed a wide range of abilities: the psychomotor skills include a very good manual abilities, a good non-verbal communication and partially expressed verbal communication, the social abilities include a good eye contact and participation in playwork and the emotional abilities concern the child's manifestation of happiness, proudness of managing with his work by himself, anger, boredom and others. We believe that a more detailed knowledge on the developmental and behavioural phenotype of CDC should take an important part of genetic counseling.

E-P05. Discovering and disclosing the family disease: stories from Huntington's disease consultands, and their partners.

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Background: Research suggests that some Huntington's disease consultands grow up knowing about the disease, whilst others do not. Indeed, some only find out after the death of a parent or sibling, or after a relative has been diagnosed. This issue is important for several reasons: individuals might be disadvantaged emotionally, socially, financially or medically by having genetic information withheld or disclosed, and conflicts may arise within families if some cannot accept a parent's or sibling's right to privacy or right not to know.
Methods: In-depth interviews were undertaken with people who have received genetic counselling for Huntington's disease, and their partners. The interviews explored how participants found out about the disease; whether subsequent relatives had or had not been told; the factors which influenced telling or not telling; who should tell; and views of the genetic counselling process.
Results: We explored how and when participants found out about Huntington's disease and the effect this had on subsequent family relationships and decisions to tell other relatives such as children. Respondents' views about whose responsibility it is to pass on this type of information were also examined.
Conclusions: The impact of Huntington's disease on family dynamics may result in family secrets and/or living at risk. The level of disclosure to relatives can at times be limited but also depends on the psycho-social, cultural and familial context. Ultimately, we hope that this study will contribute towards a wider understanding into the dynamics within families after someone in that family attends for genetic counselling.

E-P06. Adults with Marfan Syndrome: Sexual Functioning and Reproduction

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As individuals with Marfan syndrome are increasingly diagnosed prior to childbearing, there is opportunity to study factors related to the diagnosis that influence their reproductive planning. Data will be presented from an exploratory study of 174 adults with Marfan syndrome regarding their reproductive plans. A majority of survey respondents were Caucasian and well educated. Fifty eight percent were female and 64% were members of the National Marfan Foundation. Sixty percent of the cohort reported difficulties with sex drive. Age (50+ yrs), striae, back pain, and low quality of life were each independently correlated with a lack of sex drive. Forty percent of the respondents had children, with 33% having one or more children affected with Marfan syndrome. Approximately 62% of the total cohort agreed that having Marfan syndrome has significantly affected their decisions about having children. Age at diagnosis, mitral valve prolapse, and the view that Marfan syndrome has adverse consequences on one's life were each independently correlated with the perception that being affected had influenced their reproductive plans. Of the total cohort, 69% reported that they were interested in prenatal testing for Marfan syndrome. Clinical features and psychosocial issues both contribute negatively to affected adults' reproductive decision-making and sexual well-being. Genetics professionals are ideally positioned to address concerns surrounding reproduction with Marfan syndrome patients and to refer those with significant sexual or reproductive concerns for further evaluation and management.

E-P07. Caring For Fronto-temporal Dementia Patients: Influences Of Premorbid Relationship On Current Caregiver Burden

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Introduction: Fronto-temporal dementia (FTD) is a neurodegenerative disorder characterised by personality changes, alterations in social conduct, aphasia, and a decline in frontal cognitive functions. Its onset is mostly during the presenium. In 20 percent of the patients a familial form with an autosomal dominant pattern of inheritance is seen.
Research has shown that caring for a demented patient with whom the premorbid relationship was warm and positive is less stressful than if the relationship was problematic. Little is known about associations between quality of the premorbid relationship and subjective burden of the primary informal caregiver of the FTD patient.
Objective: To get insight into the caregiver burden of primary informal caregivers of FTD patients in association with the quality of the premorbid relationship.

Participants: Informal caregivers (65 in total) who were registered at the outpatient clinic of the department of Neurology at the Erasmus MC. Two groups were distinguished a) caregivers of patients that live at home, and b) caregivers of patients that are hospitalised.
Assessment of variables: Subjective burden is operationalised and assessed by:
· Emotional burden of the caregiver as measured by a section of the Neuropsychiatric Inventory on neuropsychiatric functioning of the patient.
Quality of the premorbid relationship is measured by:
· Self-report questionnaire on 1) closeness, 2) communication, 3) similarity in views about life, and 4) degree of getting along.
Results: The quality of the premorbid relation will be related to subjective burden as outcome measure. The statistical analysis to be applied is multiple linear regression.

E-P08. Psychosocial aspects of dwarf child on school age

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Introduction: The Dwarfism is a relatively frequent feature in the genetics pathology. In our study, from 2995 cases with genetics diseases, 97 (3,2%) presents Dwarfism. In all the cases, this is a specific feature of the disease or syndrome. Objective: The identification of the main psychosocial problems of the dwarf children during school age. Material and Method: 32 children on school age with different etiologies of dwarfism (achondroplasia, hypochondrodisplasia and others osteochondrodysplasias, mucopolysaccharidoses, osteogenesis imperfecta, growth hormone deficiency, Turner syndrome etc.) underwent psychological tests and sociological investigations. In every case we followed: scholar performances, community accommodation, effort adaptation, child's behavior in school, family and society. Particularities regarding age, sex, family, diagnostic, treatment, are identified in this study. The special needs of current care inside the family, medical care, psychological and pedagogic support, are evaluated. Results: All the school-age children with dwarfism have psychosocial problems. From these, the most frequent are: the problems of family adaptation, school adaptation and professional orientation. In some cases, nor the family, neither the school or the society doesn't find the best methods for adaptation to the child's sufferance.

E-P09. Behavioural phenotype in two cases of Wolf-Hirschhorn syndrome

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Wolf-Hirschhorn syndrome (WHS), a rare condition with characteristic facial traits, organ malformations, and functional impairment associated with partial short arm monosomy of chromosome 4, used to be invariably linked with profound psychomotor and mental retardation and lack of social or emotional communication. Recent data on better both somatic (eg. walking, general survival) and behavioural (eg. communication, social functioning) development in WHS give rationale to more detailed studies of the WHS phenotype. We undertook a detailed qualitative study of the behaviour profile of two children with WHS based on subsequent image analysis elicited from a video sequence recorded of a therapist-guided playwork, which was featured in the environment structured according to the principles of Maria Montessori. The focus was set on children abilities and not defects. Based on the results obtained, we attempted to elicit the needs of studied children in order to favour preventing of secondary mental impairment in children with WHS and stimulating their development by their families. Our results support the previous communications on better abilities profile of children with WHS (psychomotor, emotional, communicative, and social ones) than commonly attributed to this syndrome. We think that the growing data on the behavioural phenotype in genetic conditions, including WHS, will contribute to better and more complete genetic counseling.

E-P10. Congenital Malformations and Genetic Disorders as A Source of Investigation of the Psychosocial Repercussions in Pregnant Women in a State Hospital Prenatal Clinical Care from Rio de Janeiro, Brazil.

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We conducted an investigation of the psychosocial repercussions in high genetic risk pregnant women based upon a diagnosis of congenital malformations and/or genetic disorder at a Medical Genetics reference centre in the State of Rio de Janeiro, Brazil, during 2000. The aim of the investigation was to share the experience with 141 women, as far as comprehending the process of decision making and the necessary change of attitude needed by the families upon a clinical and/or laboratory diagnosis of congenital malformation or genetic disorder. The method used, included medical document research as to map social, demographic and cultural characteristics, ethnographic protocol (Geertz, 2000), interviews with the families, and the Life History Method (Becker, 2000), as to investigate the impact of the diagnosis. The population investigated were referred from different geographic regions of the State of Rio de Janeiro (14000000); most couples had a low schooling education; only 12% had received a genetic counselling previously the current pregnancy; the mean maternal age was 31 years old; a very low uptake of invasive exams (amniocentesis, specially) was observed due to different reasons such as emotional, moral religious or ethical (in Brazil therapeutic abortion isn't permitted by law). The maternal age risk factor was the most important one (80%). The information is the central aspect in the process of making decisions. It's necessary to create favorable psychological conditions to the understanding of the information, guaranteeing the patient's presence until the end of the process and hers return to the genetic counseling.

E-P11. What is missing from prenatal genetic counselling?

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Genetic counselling research has been criticised for focusing on outcomes rather than process (Pilnick & Dingwall 2001). There have been less than 25 published process studies since 1977 with few in the area of prenatal diagnosis. Other literature concerning prenatal genetic counselling process describes retrospective, subjective impressions of session content and decision-making processes. The process of prenatal genetic counselling remains largely unexplored. Following prenatal diagnosis parents have a choice to accept or reject the pregnancy. Conditions detected range from lethal to mildly disfiguring.
Content of genetic counselling sessions appears to be largely information giving (Michie, Marteau & Bobrow 1997) and exploration of other issues may be ignored. The continuing adherence to non -directive counselling may minimise counsellor involvement with decision-making (Terrell White 1999) and possibly constrain moral discussion for those who desire it. Research is needed to determine whether clients wish to have a level of moral discourse available to them.
As the scope of genetic screening and diagnosis increases and presents new and increasingly complex dilemmas, inclusion of a client-led moral discourse may be seen as a justifiable goal of future genetic counselling practice.

E-P12. Dinamic counselling in Haemophilia

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It is now possible to identify carrier status and to perform prenatal diagnosis of haemophilia. Therefore, the parents can choose whether to have haemophilic children or not, and this choice will condition the rest of their lives. Thus counselling is an important aspect and it should not be a once in a lifetime event: the decision-making process is something that should be on going. Counselling

should provides families the option of changing their mind. Rational aspects and doubts they might have, even month or years after the first consultation, must be considered. Nowadays it is possible to get information easily from several sources even though it could be inaccurate and contradictory, counselling allows people to discuss and to exchange views concerning medical progress.
Drawing up a counselling outline decreases the disagreements regarding the medical and psychological aspects. If all the centres provided the same information, the perception, about the discrepancies among the various specialist would decrease.
Counselling should be geared to the subject we are talking to, and should take into account social and cultural levels, age and reasons for counselling. The counsellor must be neutral and all the possible strategies must be considered.
The basis for a long lasting therapeutic alliance can be achieved through counselling; the main advantages can be had by the patients and their families as by the staff. The type of dynamic counselling we are suggesting needs to be up-to-date. Everyone working in genetic counselling must be involved, from laboratory staff to the consulting physician.

E-P13. Attitude of Italian Haemophilia Carriers towards reproductive choices

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Haemophilia A and B are X-linked bleeding disorders due to coagulation Factor VIII or Factor IX deficiency.
Severe haemophilia is characterised by life-threatening bleeding, chronic arthropathy and recently HIV and hepatitis virus infection. Progress in therapy and prophylaxis has improved the quality and expectancy of life of the haemophiliacs.
Many women at risk request genetic counselling and prenatal diagnosis. To investigate carriers' knowledge about treatment and prenatal diagnosis we designed a multichoice questionnaire.
So far, 189 women, most of them aged between 20 and 45 years, from all over Italy have answered.
Regarding the genetic aspect, in familiar cases, 22% claim they found out about the risk of having an affected child only after a haemophilic son was born and 30% did not answer the question.
285 pregnancies are reported for the 173 women, and 33 PD were performed. One of the 3 prenatally diagnosed affected males was aborted.
Many couples choose not to have other children after the birth of a haemophilic, mainly due to their carrier status.
The availability of new and safer concentrates has improved the way people feel about of the disease and therapy.
In 20 years experience at the Gaslini Institute, 117 PD were performed and 87% of the affected males were aborted. We suppose that the couples requesting PD had already decided to abort the affected males. Many carriers avoid pregnancy since having to make a decision on abortion is unbearable for them.

E-P14. Predictive testing for BRCA1/2: attributes, risk perception and management in a UK multi-centre clinical cohort

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The aim of this multi-centre UK study is to examine the attributes of a cohort offered predictive genetic testing for breast/ovarian cancer predisposition. Participants are adults unaffected with cancer from families with a known BRCA1/2 mutation. This is the first large multi-centre study of this population in the UK. The study evaluates mental health, perceived risk of developing cancer, preferred risk management options, and motivation for genetic testing. Participants were assessed when coming forward for genetic counselling prior to proceeding to genetic testing. 312 individuals, 76% of whom are female, from 9 UK centres participated in the study. There are no gender differences in rates of psychiatric morbidity. Younger women (<50 years) are more worried about developing cancer than older women. Few women provide accurate figures for the population risk of breast (37%) or ovarian (6%) cancer but most think that they are at higher risk of developing breast (88%) and ovarian (69%) cancer than the average woman. Cancer related worry is not associated with perceived risk or uptake of risk management options except breast self-examination. The findings indicate that younger women may be particularly vulnerable at the time of the offer of a predictive genetic test.

E-P15. Breaking the rule of nondirectiveness: the psychological impact of early identification of breast cancer patients at risk for hereditary breast cancer

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Past five years are characterized by a steady growing interest in genetic counseling for hereditary beast cancer. Applicants for genetic counseling have mostly concerned healthy persons at high risk for developing breast cancer; they were well motivated and highly educated. By identifying cancer patients with a BRCA gene mutation and subsequently taking preventive measures and diagnostic procedures, life expectancy of patients and relatives may improve. Besides healthy at risk persons, breast cancer patients will apply, even during course of their treatment. Currently breast cancer patients are stimulated to apply for genetic counseling in a fairly directive manner at the Department of Radiotherapy of the University Medical Center Utrecht in the Netherlands. The protocol used consists of the following consecutive stages: 1) screening on eight factors predisposing hereditary breast cancer, 2) composition of a pedigree, 3) genetic counseling and 4) DNA analysis. Aim of this study is to gain insight into psychosocial consequences of this directive approach and provide guidelines for a directive approach in genetic counseling. Effects will be investigated using questionnaires focussing on psychological wellbeing as well as knowledge and attitude towards genetic counseling. Subsequently a semi-structured interview will be administered.
Genetic risk assessment while starting radiotherapy treatment may prove a psychological burden. However, we expect patients who are informed of an unlikely genetic cause will also be relieved. Based on previous studies indicating decreased psychological wellbeing of patients declining genetic risk assessment we expect that patients who withdraw from the protocol will report less psychological wellbeing than others.

E-P16. Predictive testing for hereditary breast and ovarian cancer (HBOC): Psychological impact and health related behaviour in the year following the communication of the predictive test result.

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Since the end of 1997, predictive genetic testing for HBOC has been available as a clinical service in Leuven. Test applicants are offered pre- and post-test psychological counselling in the context of a multidisciplinary approach. Longitudinal psychological research, embedded in the clinical service, is aimed at establishing a baseline evaluation of the testees and at assessing the psychological impact of predictive genetic testing. In the present paper, follow-up data for the first year after predictive testing are discussed for 38 tested women (21 carriers and 17 non-carriers).
The mean general distress levels of carriers and non-carriers were not significantly different during the pre-test period nor one year after predictive testing. Moreover, distress levels were not higher (some even lower) than in the general population. Except for the STAI-State score that decreased over time, no significant differences were found between pre- and post-test measures.
Most of the carriers who opted for regular medical examinations followed the screening recommendations. About 10% of the carriers had a prophylactic mastectomy within the year after the test result. More than two thirds of the remaining group of carriers stated that

they would not have a prophylactic mastectomy in the future. Of the carriers above 35 years, 37% already had an oophorectomy before applying for predictive testing whereas 44% had prophylactic oophorectomy after genetic testing.

E-P17. Acceptance of testing for genetic predisposition to breast cancer: Part 2 - The attitude of society

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Background: This study represents the second conclusive part of a research project.
Objectives: To assess the attitude of the society towards the testing of healthy people for genes predisposition to cancer and summarize the results of the two parts of the project.
Setting: The study was carried out with the participation of 15 students - molecular biology, 15 women at risk and 30 healthy women tested by means of a personal interview and a psychological questionnaire.
Participants: Total 109 women (aged 21 - 73): 49 medical professionals and 60 others.
Results: 27 (25% of participants) refused to collaborate; 23 (28% of the collaborating ones) reported personal experience or positive family history of breast cancer. 67% of the students were familiar with BRCA 1 and 2. 75% of the participants would accept pre-symptomatic breast cancer testing, if required.
Conclusions: There is no strong correlation between age and attitude as well as between personal tragic experience and attitude. However, there is a strong positive correlation between educational level and level of knowledge about cancer. Thus the need of more information is clearly indicated.

E-P18. BRCA1/2 Testing in Hereditary Breast and Ovarian Cancer Families: Impact on Relationships

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Members of Hereditary Breast and Ovarian Cancer (HBOC) families often express concern within genetic counseling about the impact of BRCA1/2 testing on relatives. Yet, the impact of the decision to undergo cancer susceptibility testing and/or the results themselves on family relationships is not known. Within a randomized trial of cancer genetic counseling method, 212 members of thirteen HBOC families were offered BRCA1/2 testing for a known family mutation. Data on family functioning was collected at baseline and again at 6-9 months following the receipt of test results, or at the equivalent time for those participants who chose not to undergo testing. 181 participants elected to undergo genetic testing (85%) and 47 (26%) were identified as mutation carriers. The Family Relationship index was used to measure changes in perceived cohesion, conflict and expressiveness from baseline to follow-up. Change was analyzed for each measure between testers and non-testers and between those who tested positive and negative. Overall, there were positive changes in expressiveness and cohesion and negative changes in conflict. Individuals who opted for testing had lower feelings of cohesiveness and expressiveness than those who declined testing ($p = 0.054$ and $p = 0.09$ respectively). Expressiveness was significantly lower in those who tested positive rather than negative ($p = 0.005$). Results suggest that choosing to undergo BRCA1/2 testing and living with positive results may cause less disruption in families in which there are not strong feelings of cohesiveness or expressiveness.

E-P19. Referral patterns for diagnostic genetic testing in Huntington's disease.

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Ethical guidelines for HD predictive testing are widely observed and appear to work well. However, genetic testing can also be used to confirm or exclude HD from the differential diagnosis in apparently affected individuals. There are no guidelines for 'diagnostic' testing, but anecdotal reports suggest considerably more problems than with presymptomatic testing. Some UK laboratories require signed consent for diagnostic testing, but practice varies considerably between centres.
258 requests for HD diagnostic testing were received by the molecular genetic laboratory in Manchester between 1993 and 2000. The number increased steadily from 18 in 1993 to 48 in 1999. Referrals originated from 33 clinicians in 8 specialties. The percentage of positive test results varied between clinicians from 38% to 100%. Logistic regression analysis indicated a significant difference between referring clinicians ($p=0.0006$) but no significant effect due to specialty. Requests from clinical geneticists declined from 90% in 1993 to 40% in 1999, while the proportion of positive results decreased from 88% to 62%. Clinical information was provided on 60% of request cards, and family history on 45%. Referring doctors with a low rate of positive results presumably use the test to eliminate HD in cases with relevant symptoms but no family history. The use of consent forms in such cases may arguably raise patients' anxieties unnecessarily, but conversely may help to reduce the risk of performing 'diagnostic' tests on presymptomatic at-risk individuals who have not been referred for genetic counselling. The implications for future policy will be discussed.

E-P20. Psychological impact of pre-symptomatic genetic testing for Machado-Joseph disease: preliminary results.

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The aim of the present study was to measure the impact of pre-symptomatic genetic testing for Machado-Joseph disease, at the beginning and end of our program of genetic counselling and psycho-social evaluation and follow-up.
We studied the first 19 individuals at-risk (1 male, 18 females; mean age: 41.7 years), to have completed our protocol. The scores of *depression* and *anxiety*, reached through the application of two scales (*Clinical Evaluation Inventory of Depression,* Vaz Serra & Abreu, 1973 and *Zung's Anxiety Scale*, Vaz Serra *et al.*, 1982), were chosen as indicators of the emotional state to of each subject and taken three moments: before pre-symptomatic testing, and at three weeks and at nine months after disclosure of the results of the test.
Our preliminary data shown that, (1) the scores of depression and anxiety did not achieve pathological levels at any moment, for the vast majority of subjects; and that (2) awareness of the test results does not cause a negative psychological impact - mean scores of depression and anxiety by the third moment were always lower than those at the first moment of evaluation. This pointed to psychologically healthy adaptation to the new genetic status (carrier or non-carrier), for all subjects studied, even in those cases where anxiety and/or depression raised immediately after testing (three weeks). Despite this small sample, the results are in concordance with previous studies and helped us to delineate future research.

E-P21. The revelation of the Huntington's disease asymptomatic diagnosis : psychosocial aspects

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The main purpose of this research is to study how to minimize the psychological dangers of an asymptomatic genetic diagnosis of Huntington's disease. Therefore, we record and study the conversations that took place in the predictive consultations. We study it with a theory which updates the social and cognitive aspects of the verbal interactions : the Interlocutory Logic. This analysis allows us to show where the complexity of these interactions lie and to pinpoint very subtle conversational mechanisms which are implicated. More precisely, we show how the interlocutors-in-interaction (practitioners as well as the asymptomatic individuals at risk) refer to the demand of knowledge during the successive dialogs that are initiated by it.

E-P22. Predictive medicine: gynaecologists' opinion survey

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To author's opinion there is the deepest gulf between the highest level of the modern molecular medicine and its understanding of the physicians and persons. The aim of this investigation consists of the study of gynaecologists' views on some problems of the predictive medicine. Materials and method. At the beginning of six-hour lecture, 50 of obstetricians and gynecologists (41 women, 9 men aged from 24 to 53 years) got a self-completing questionnaire with 6 questions. Results. 1st position: Genomic dactyloscopy is a modern method for the investigation of dermatoglyphic patterns (Yes-90%). 2nd position: I'd like to be examined by technique of genomic dactyloscopy (Yes- 52%). 3rd position: I am informed about predictive medicine (Yes - 10%). 4th position: I'd like to have the information on my gene mutations predisposing to some common diseases (Yes -34%). 5th position: Do you want to have your genetic certificate? (Yes - 28%, all of them are the younger generation of doctors). 6th position: What do you think about ethical, legal and social aspects of predictive medicine? (Absence of problem -2%, confidentality-92%, privacy - 96%, dread of cloning - 5%, questions of financing - 80%) These findings like our previous reports corroborate the low level of genetic knowledge of our doctors and citizens. It is very important to create special programme to educate our population on medical genetics and predictive medicine.

E-P23. Life events and psychological risk in individuals entering the protocol of pre-symptomatic testing for FAP-TTRMet30.

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Life events may have an important impact on psychological risk of persons who are at genetic risk for a deleterious inherited disease. We made a qualitative analyses of psychological interviews of 23 individuals at-risk for familial amyloid polineuropathy (FAP), type I or TTRMet30, the first to enter our protocol of pre-symptomatic testing for this disease. Thus, we chose 4 categories of history of life events that might influence the present psychological conditions of individuals at-risk: (1) separation (emotional separation from significant others); (2) change (change of environment - school, home, other - e.g., due to the death of an affected parent); (3) contact (contact with patients and knowledge derived about the disease); (4) care-giving (taking care of an affected relative). We hypothesised that those events of personal history may turn them more fragile, increasing their psychological risk during the pre-symptomatic testing process, as measured by the "Psychopathological Symptoms Inventory" (BSI) (Derogatis, 1982). The 'psychosis index' varies significantly ($F(1,22)=4,169$, $p<0,05$) with the experience of separation (1); the 'phobia/anxiety index' varies significantly ($F(1,22)=7,998$, $p<0,01$) with the experience of change (2); the 'positive symptom index' ($F(1,22)=6.297$, $p<0.02$) and the 'somatisation index' ($F(1,22)=6.924$, $p<0.02$) both vary significantly with the experience of care-giving of that person (4). Contact did not influence any indices studied. In conclusion, some life-history events seem to influence negatively the present psychological condition of individuals at-risk and may be an indication for being more cautious in the procedures of the pre-symptomatic testing protocol, namely in delivering test results to those individuals.

E-P24. Adverse effects of predictive testing for Huntington disease underestimated

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Objective
In general, studies on psychological consequences of predictive testing for Huntington disease (HD) revealed few harmful consequences for identified gene-carriers and little relief in non-carriers. Little attention has been paid to the characteristics of individuals who were lost to follow-up, as it is difficult to get access to this group. Therefore, the adverse effects in carriers and non-carriers may be underestimated.
Subjects and methods
In the long-term follow-up of predictive testing for HD 180 test applicants completed questionnaires which assessed future expectancies (Beck Hopelessness Scale), intrusion of adverse ideas and denial-avoidance reactions (Impact of Event Scale) and general well being (General Health Questionnaire).
Results
After receiving the DNA test result, 39 persons (22%; 18 increased risk, 21 decreased risk) did not return for follow-up and additional counselling (dropouts). After 7-10 years, about 69% is lost to follow-up. There were no differences between non-carriers who did return (participants), and dropout non-carriers. Dropout carriers had reported at pre-test significantly more hopelessness ($F_{(1,76)}=6.5$; $p=.013$), more intrusive thoughts about HD ($F_{(1,76)}=13.4$; $p=.000$), more avoidance behaviour ($F_{(1,76)}=10.1$; $p = .002$) and a worse sense of well-being ($F_{(1,76)}=7.0$; $p=.010$), than participating carriers.
Conclusions
People who are vulnerable to psychological distress may have reacted with extreme denial of an unfavourable test outcome and avoidance of the messengers of the bad news (geneticists) and their associates (psychologists-researchers). Oral information by relatives supports these speculations. Studies reporting few harmful effects by identified carriers may have underestimated the real impact of the test results.

E-P25. Social and Ethnic Differences in Attitudes and Consent to Prenatal Testing

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Introduction:
This paper will present details of an ongoing ESRC/MRC funded study comparing the attitudes of different social and ethnic groups to prenatal testing. The findings from the pilot study will also be presented.
Background:
Advances in DNA technology mean that a wider range of prenatal tests will soon become available. Obtaining separate informed consent for each condition is likely to cause confusion and provoke considerable anxiety amongst parents. A classification system is therefore required and needs to be built upon parents' attitudes to testing and their perceptions of the similarities and differences between conditions.
Research Design:
The attitudes of several hundred postnatal Pakistani and indigenous 'white' women towards prenatal testing, for a range of conditions, are being compared using quantitative and qualitative data. The effect on attitudes of educational level is also being assessed. The acceptability of the classification system will be assessed amongst maternity service users, providers and voluntary organisations.
The pilot study assessed women's ability to manage descriptions of 30 conditions ranging in severity and age of onset. Twenty-one women completed the questionnaire and considered whether they would a) want a prenatal test for the condition and b) consider a termination of pregnancy if the test was positive. The results from these 21 participants will be presented.

E-P26. Pakistani families' reactions and attitudes towards ambiguous genitalia

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The birth of a baby with ambiguous genitalia is considered both a medical and a psychosocial 'emergency'. Sex assignment in a newborn with ambiguous genitalia requires multidisciplinary team effort. Reactions and attitudes to gender ambiguity may vary from culture to culture. The lack of understanding about parents' attitudes and cultural differences may lead to misconceptions, affecting the desired outcome of such cases. Between 1997 to 2001, 44 cases of genital abnormality were referred to the Yorkshire Regional Genetics Service for chromosome analysis. Twenty of these cases were of

Pakistani origin. This paper highlights the reactions and attitudes of two Pakistani families, which was observed during genetic counselling. In the Pakistani culture intersexuals are categorised as 'Hijras' - a third sex or a third gender. They are not socially accepted in the wider Pakistani society. These individuals lead their life as outcasts, in their own socially isolated community. This paper will show how intersexuals are conceptualised in the Pakistani culture. This paper will also highlight an ethical dilemma arising from a request of termination of pregnancy from one family with genital ambiguity. The implications for genetic counselling arising from these cultural differences in attitudes towards ambiguous genitalia will be discussed.

E-P27. Genetic counselling in oncology: the role of the social worker

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In this presentation we share our experience in the decision-making process of counsellees who present themselves with hereditary breast and ovarian cancer (HBOC). Individuals at high risk for HBOC are faced with a number of complex decisions concerning taking (pre)symptomatic gene mutation testing. Visits to the social worker provide assistance during this process.
Studies show that issues to be addressed during these visits, include perception of cancer risk, consequences of DNA testing and compliance with screening. However, these studies do not indicate which tools for psychosocial support may be used. Therefore, we have developed an approach which includes assessment of psychosocial concerns, the individual's phase of life, relationships with family members, social networks and present coping strategies. Psycho-educational interventions and methods for empowerment are used and focus on highlighting various perspectives of the problem. This approach aims to prevent negative effects to psychosocial well being.
Since 2000, 512 counsellees have been seen. Most of them are emotionally healthy individuals, suddenly faced with the burden of a genetic predisposition and/or a possible treatment for cancer. The family situation may also be burdensome due to existing cancer or a recent cancer related death in relatives. Combining the issues extracted from the literature with our approach of addressing psycho-social concerns has resulted quite consistently in a well-balanced decision to take a screening test or to postpone the test, being emotionally prepared to accept the consequences in both cases. We suggest that our approach to decision making may prevent psychosocial problems in counsellees.

E-P28. Health in intellectual disabilities people of more than 40 years old.

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Life expectancy in intellectual disability people is increasing as in normal population. In Down syndrome, it was 9 years old in 1930, and now more than 60.
This increasing life expectancy is in relation to a better medical care during infancy, but ageing results in others diseases and in important social problems. We compared health status of three groups of patients with intellectual disability older than 40: group A: Down syndrome (n=112), group B: others chromosomal abnormality (n=7), group C: unexplained intellectual disability (n=25). Social data were compared. These data are retrospective from their medical records. Results: As expected, medical and psychiatric complications were more frequent in group A; the observed diseases were in descending order: hypothyroidism, cataract, depression, and epilepsy. Some diseases were seen only in this group (autoimmunity, keratoconus). Thromboembolic diseases were more frequent than expected (5%). From social data analysis, most of those patients were living in their own family, and institutionalisations often happen in an emergency context. To adapt one's care to one's personal needs, we created a special document, "the life notebook" to summarise important information (medical, social, personal...); we present it.

E-P29. Management of women with a family history of breast cancer in the UK North West Region: training for implementing a vision of the future

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Specialist genetics centres in the North West region of the UK, as elsewhere, have experienced an exponential growth in demand for genetic counselling services for women with a family history of breast cancer. We report a strategy to devolve moderate risk breast cancer genetic counselling to the cancer unit level through an integrated education programme for specialist nurses. The training programme and follow-up support for the clinics are described, and evaluation of both the training programme and the pilot breast unit Family History Clinics shows that outcome criteria have been met in all cases. The training includes didactic teaching, role play and clinical experience. To date, 21 nurses have been fully trained, and 5 breast units are now running regular dedicated breast cancer Family History Clinics. A Patient Satisfaction Survey indicates that women with breast cancer family histories are satisfied with the service provided. A limited trainee evaluation has enabled some improvements to be made to the course, and to the follow-up support provided to nurses running their own Family History Clinics.

E-P30. Facilitated peer group supervision for genetic counsellors: one year's experience.

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There is an increasing awareness of the potential value of supervision for genetic counsellors (Kessler, 2000). Kennedy (2000) has outlined the merits of different models of supervision: individual, in a peer group, or in a facilitator-led peer group. At the present time it is not routine practice in the UK for genetic counsellors to receive funded supervision during working hours. As experienced genetic counsellors we set up a facilitated supervision group to provide challenge, new dynamics and structure in supervision. This voluntary supervision group of between 6 and 10 genetic counsellors has met monthly for 1 ½ hours over the last year in a neutral environment, facilitated by a humanistic counselling supervisor with an interest in group supervision and no prior experience of genetic counselling. In this paper, we will describe the process of setting up the group, who to include, group size, choosing a supervisor, setting a contract, defining boundaries and aims and anxieties of the group. We will report on the themes that have emerged and evolved over time reflecting the experiences of the group and its growing maturity, from both the supervisees' and supervisor's perspectives.

E-P31. Email as a communication tool for patients with genetic diseases: the Orphanet experience

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Web sites and email are nowadays important resources that provide access to medical information on genetic diseases and advice that patients and health care professionals may otherwise never receive. Our experience in this area is based on Orphanet, a European internet-based information database on rare diseases which has a growing readership now averaging over 75 000 individuals each month, half of them being patients or their relatives. Orphanet receives an average of 65 unsolicited emails each month with requests for medical information and advice. We categorised the e-mails according to whether responding according to a predetermined strategy would pose any ethical, professional or legal problems and which type of response would be most appropriate. All unsolicited e-mails received between January 1, 2001 and June 30, 2001 were included in the audit. Using a general strategy would pose a problem in only 3% of cases and 9% fall into a "grey zone". With regards to the strategy, the majority of the e-mails could be answered using a

general response (84%), 7% would warrant an explicit suggestion to see a physician, 7% would require some degree of medical expertise to respond, and 2% remain in the "grey zone" with regards to how to respond. Producing a strategy for responding to unsolicited emails has allowed Orphanet to continue to orient and educate database users while reducing the number of difficult dilemmas, reducing the workload involved, and most importantly, protecting patients by advising that they seek proper medical care in person from qualified professionals.

E-P32. Development of the profession of genetic counselling in Europe

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Genetic services are established throughout Europe, although levels of service vary and some have minimal input from non-medical professionals (genetic nurses or counsellors). Skilled health professionals are increasingly needed to interpret the benefits or otherwise of genetic advances for the health of individuals. Non-medical genetic counsellors can provide services and so enhance access and equity for the population. To ensure safety, standards of professional practice must be established. In the United Kingdom (UK) and The Netherlands, formal processes have been established to ensure genetic counsellors can deal with the complex scientific, ethical, social and psychological issues involved in genetic counselling.

In the UK, a profile of genetic counsellors in current practice has provided a baseline for assessment of the new registration process. Of 150 counsellors who provided data on their current post, professional background, educational preparation and ongoing training needs, 83% are educated to degree level, and 77% are registered nurses or midwives. Fifty-three percent have a generic post in a genetic centre, while 24% work in disease-specific posts. Of concern is the finding that 32% consider they have current unmet educational needs. Sixty-nine percent intend to register formally as genetic counsellors.

The experiences of individual countries should contribute to building a platform for the development of the genetic counselling profession in Europe. Although health systems, education and cultural differences make a formalised Europe-wide registration system impractical, it may be possible to develop European guidelines for good practice that are adaptable to the needs of each country.

E-P33. Unintended Messages: the ethics of teaching genetic dilemmas

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The teaching of bioethics often uses challenging cases. However, these cases may cause harmful messages to be received by specific communities. We present as an example the "Case of Dwarfism," developed shortly after the discovery of the common mutation for achondroplasia in 1994. This case describes a couple, both affected with achondroplasia and expecting a child, who plan to terminate the pregnancy if prenatal diagnosis reveals the fetus is of average stature. It is often employed as a teaching case for US health professionals, designed to stimulate debate over their role as the gatekeepers of access to genetic testing. It also compels audiences to examine their views on reproductive freedoms and the limits of parental autonomy. While this case may challenge stereotypes about the appropriate uses of prenatal diagnosis, it potentially fosters a more subtle stereotype about the community it intends to serve. It presents people with achondroplasia as motivated to make reproductive decisions based merely on perceptions of their physical features. Yet, a recent study of people affected with achondroplasia revealed that the majority considered knowing the prenatal diagnosis of average stature unimportant and that only 2% would consider termination based on this finding. Using this case to challenge the view of what makes for a "healthy baby" may actually deny that those with achondroplasia simply share the common parental desire for healthy children. While such cases remain important teaching tools in genetics, we should be aware of our own inadvertent messages and their potential for creating discriminatory attitudes.

E-P34. The Manchester MSc genetic counselling programme: ten year's experience

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We report our experience of running the first Masters level genetic counselling course in Europe. The programme provides vocational training for genetic counsellors and has contributed to psychosocial genetic research. The students and staff have helped to inform the evolving genetic counselling profession in Europe, including advice to new training programmes and defining requirements for genetic counsellor training and registration. The two year course initially had an annual intake of 4-6 students, but has now expanded to take 8-10 new students each year. 62 students have enrolled on the course (including 14 current students); while most students have a relevant first degree, they also have varied backgrounds, including nursing (18 of 62), community based health or social care experience, and education. 47 students have completed the course to date. Of these, 37 are currently working as genetic counsellors (30 in the UK, 1 in Ireland, 6 outside Europe), 5 are working in genetic counselling research, 2 are completing further academic qualifications, and 3 are not currently employed in genetics. Although the science of human and clinical genetics is an important aspect of the training, a psychosocial approach to genetic counselling practice and research is promoted. This is reflected in the choice of research project with the majority choosing qualitative studies of patients/families' views and experiences. Collectively the students' projects have produced a significant contribution to the body of genetic counselling research, through publication and presentation at scientific meetings including past EMPAG meetings.

E-P35. Setting up a genetic counselling service for the Irish traveller population

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The Irish traveller population are a distinct ethnic minority group with origins going back to the 12th century. They are a small minority group, making up 0.5% of the Irish population (approximately 25,000 people), however, as they have a history of intermarriage, there are at least 30 autosomal recessive conditions seen more prevalently in the Irish traveller population than the general population . As with other ethnic minorities, genetic health professionals must be sensitive to the social and cultural values of a particular ethnic group before genetic counselling to ensure the accessibility and suitability of their service. With this in mind, a working group was set up to evaluate the need for such a service, the accessibility of the current service and factors which may influence uptake of such a service. We, together with representatives of the Irish traveller organisation, other medical professionals and the clergy, have created a development plan to address problems with the need, accessability and suitability of our genetic counselling service. Problems highlighted included maintaining cultural norms, preliteracy, nomadic lifestyle, distrust of authority and preserving individual autonomy against a background of strong family unity and strong Roman Catholic beliefs.

E-P36. Genetic Education and Counseling in Mental Retardation at Primary Health Care Level - Preventive Strategy in India.

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Rapid advances in medical genetics and DNA technology in developed countries have emerged with better understanding of the causes and therapy of several genetic disorders. Yet in India, genetic diagnostics is misperceived as expensive, and concerned with rare disorders, partly due to the limited resources and technically skilled personnel.

CREMERE - a tertiary Genetic Centre therefore organized genetic education to community heath volunteers (CHVs) (N>400), working at primary health centres located in slum, semi-urban and rural areas. The training covered detection of mental retardation, causes

(environmental and/or genetic), genetic tests, recurrence risk and pregnancy monitoring with reproductive options.
It was surveyed that parents were largely unaware about nature of the genetic disorders, hereditary factors and existing genetic diagnostic services. The trained CHVs successfully detected the MR cases with genetic factors and told the parents about the training and rehabilitation aspects. Monitoring in future pregnancy using triple marker screening and fetal ultrasound were explained. The concept of prenatal diagnosis, yet not evolved, could be integrated at the community level.
We encountered various barriers like religious and social misconcepts, family traditions and cultural beliefs. Lack of genetic team approach, sophisticated laboratory infrastructure, technically skilled genetic personnel and unawareness of existing genetic services was perceived as the main constraints.
Genetic counseling done in a few cases depicting the different socio-economic, emotional and medical problems, and measures adopted to overcome these will be discussed. Integration of genetic services for prevention of genetic disorders associated with mental retardation was feasible through this community education attempt.

E-P37. Joint research on the problem of colourblindness. A study in Calabria and Basilicata (Continental Southern Italy)

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A study of colour-blindness carried out in Calabria and Basilicata (Continental Southern Italy) was based on three disciplines: biology, psychology, and pedagogy. In all, 63,933 students (age range 11-14 years) of both sexes underwent the Ishihara test in order to identify this visual anomaly. Both Calabria and basilicata showed mean frequency of 4.8%, which was evaluated on 32,322 males. Calabria demonstrated a North-South decreasing trend of frequencies, according with the geography of the three ancient provinces: Cosenza (CS), 5.6%; Catanzaro (CZ), 4.6%; and Reggio Calabria (RC), 3.9%. The frequencies were significantly different among the three provinces: CS vs. CZ, $p<0.001$; CZ vs. RC, $p<0.025$; CS vs. RC, $p<0.0005$. The answers to a psychological questionnaire (18 questions) from 831 colour-blind students were significantly different from those of the 34,309 orthocromatics chosen according to statistical criteria. Statistical quantitative analyses were made by Kruskal-Wallis test, Friedman test, and Sign test; qualitative statistical analyses were made by chi square test, and that of McNemar.
A pedagogical questionnaire (13 questions) of 3,082 teachers of different disciplines confirmed their knowledge of colour-blindness as an anomaly (39%), with 58% confirming their knowledge came only through hearsay, while the rest admitted to not know anything about colour-blindness
Research financied by Fondazione Cassa di Risparmio di Calabria e Lucania (Cosenza)

E-P38. Psychological and Neuropsychological Findings in Adults with the Premutation and Full Mutation for Fragile X Syndrome

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Emotional and neuropsychological test results will be presented from a three-year genotype-phenotype study of families with a member diagnosed with Fragile X Syndrome. Variables were collected from American (N=172) and Australian (N=109) adult subjects. Subjects were administered a battery of cognitive, emotional, and neuropsychological measures. Family members found to have neither the premutation or full mutation for Fragile X served as controls. Family members who carry the premutation or full mutation for fragile X have been clinically described to manifest a number of psychological symptoms. These observations are supported with statistically and clinically significant results. Data from two ratings, a self-report and a spouse report were acquired from two instruments, a checklist and a clinical interview. Significant elevations ($p<.05$) were found in various subject groups for phobic-anxiety, anxiety, psychoticism, depression, social isolation, introversion and obsessive-compulsive symptoms
Psychological factors as a function of age were also statistically analyzed. For the premutation male group, symptoms of anxiety, phobic-anxiety, depression, general distress, and obsessive-compulsive symptoms increase significantly ($p<.05$) with age. Age-related changes in symptoms were not found in other subject groups. Neuropsychological results will be presented on a group (N=8) of older premutation males, aged 62-79. These men have significant executive functioning impairment. Some have neurological difficulties that include intention tremor. A younger group (N=18) of premutation males, aged 28 to 63, will also be presented. A subset (N=6) of these younger men also have significant executive functioning deficits. They do not have other neurological difficulties such as an intention tremor.

E-P39. Analysis of ADHD Subtypes in Fragile X Syndrome

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Research in the area of Attention Deficit Hyperactivity Disorder (ADHD) has emphasized three distinct subtypes including the Inattentive Type, the Hyperactive/Impulsive Type and the Combined Type. Within the Fragile X (FXS) research, the majority of boys with the full mutation exhibit significant attentional problems with hyperactivity, while females are more frequently described as having attentional problems without hyperactivity. The profiles of males and females with the premutation and mosaic status have not been documented.
118 individuals affected by FXS were assessed for ADHD using the SNAP-IV symptom checklists obtained through spouse report or parent report. Data was divided into comparison groups with regard to gender and DNA status. Analyses were conducted to document and describe the subtypes as they relate to gender and DNA and to determine individual patterns of ADHD subtypes.
Limited numbers of premutation females and males, and mosaic males met clinical criteria for ADHD. Premutation female inattentive scores were significantly greater than hyperactive/impulsive scores. [n=37; (p=.009)], while premutation males inattentive scores were not significantly different than hyperactive/impulsive scores [n=15; (p=.11)]. Mosaic males did not demonstrate a significant difference between subtypes. [n=13; (p=.08)]. A high percentage of females and males with the full mutation met clinical criteria for ADHD. Females and males with the full mutation demonstrated higher inattentive scores than hyperactive/impulsive scores: females [n=20; (p=.0004)]; males [n=27;(p=.0001)].

E-P40. Emotional well-being after a termination for abnormality: the impact of obstetric and social factors

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This paper will use data collected from 148 women during interviews and through postal questionnaires in the year after they made the decision to terminate a pregnancy after the diagnosis of a fetal abnormality. Most of these parents were at no particular risk when they became pregnant and malformations were discovered as a result of routine antenatal care. The relationship between scores on measures of grief and depression and obstetric, social and personal characteristics has been investigated using multivariate methods. The grief response of mothers is variable but overall, emotional well being improved over time. The principal obstetric variables, gestational age, method of termination and severity of malformation were not related to emotional well being at any time in the year following the termination. We have found no relationship between

aspects of care during and after a termination and women's subsequent feelings. Aspects of how the diagnosis was made, specifically satisfaction with getting the diagnosis and ease or difficulty of getting a diagnosis were independently related to mothers' feelings after a termination. The emotional support that women perceived they were getting from their partner was strongly related to mood both in the short and long term after a termination. A year after a termination, there was a significant relationship between subsequent pregnancies and women's feelings.
The implications of these findings for the care and support of women undergoing a termination for abnormality will be discussed in this paper.

E-P41. Attitudes towards genetic testing in a representative German sample. The influence of sociodemographic characteristics

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Background: Pre- and postnatal genetic testing have become a widely used means for the assessment of individual risk for hereditary diseases. In Germany, virtually no empirical data concerning attitudes towards gene tests have been available, so far.
Purpose: In order to explore the attitudes of the German public, we conducted a survey regarding general attitudes towards genetic testing in a sample representative for the German population (N = 2.076).
Methods: We used a subset of 13 statements out of a larger questionnaire set used in a Finnish survey. The items related to approval, disapproval, and concern for genetic testing.
Results: The results reveal that there is wide acceptance of genetic testing in the German population, about two-thirds hold a positive view. Yet, possible disadvantages are also recognised. A factor analytic analysis of the items yielded three distinct factors (approval, disapproval, concern for genetic testing). The MANOVA model revealed differences concerning attitudes towards genetic testing between various sociodemographic groups. The clearest differences emerged for religious affiliation. Members of a religious group consistently indicated a less favourable view of gene tests. Furthermore, subjects who had attained a higher educational level showed ambivalent attitudes, i.e. they scored higher on both approval and disapproval.
Discussion: In general, there is widespread support for genetic testing in the German population, but the approval of gene tests is not as high as in Finland. Similarly to a Finnish general population study, we found influences of sociodemographic characteristics on general acceptance of gene tests.

E-P42. Measurements of Breast Cancer Risk Perception: An Ongoing Study in the General Public.

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Breast cancer risk perception is an important predictor of the intention to obtain predictive genetic testing for breast cancer risk. However, many women tend to overestimate their own risk. To understand this bias it is necessary to take problems of measurement into consideration. Many studies found that open-ended probability scales lead to an increased use of the 50% option, maybe as an expression of having difficulties with understanding and using probabilities. This could lead to problems in the comparison of empirical (i.e. Gail) and personal risk estimates.
In the context of the ongoing study AttRisk a random sample of women of the general public recieved an information letter. Women completed a brief telephone survey that assessed eligibility criteria and risk status. Eligible participants were mailed a longer questionnaire and a consent form with a return envelope. Four different measurements were used for breast cancer risk estimates: a) opend-ended probability scale b) visual analog scale, c) 7-point-scale and d) 5-point-scale to assess the personal risk in comparison to peers. Additionally, they were asked how certain they were in each judgement.
To date, data from 218 respondents has been collected (response rate: 59%). About 84% were women with neither FDRs nor BC. About 20% reported a personal risk of 50%. There were strong positive correlations between the measurements (a-c) except for the comparison scale (d). Respondents reported a moderate level of certainty for each response mode. They felt more certain in using the comparison scale compared to the other measurements.

E-P43. Understanding inheritance: kinship connections and genetics

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Discussions of lay and professional scientific knowledge suggest a wide range of possible interactions between people's existing understanding of particular situations and those they may draw on from scientific work (Wynne, 1991). Lay ideas about inheritance may be developed early in childhood in the context of the family and grounded in concepts of kinship, reinforced by everyday social activities and relationships. Such ideas might be particularly resistant to change and may conflict with Mendelian explanations, making the uptake of these scientific accounts of inheritance difficult (Richards 1996, 1998, Richards and Ponder 1996). This hypothesis has implications for genetic counselling, the teaching of genetics and public knowledge in general.
The hypothesis is being investigated in a qualitative study of young peoples' connections between concepts of inheritance, genetics, family and kinship. Interviews use open questions, which encourage participants to explore, in their own words, the concepts of inheritance, genetics and kinship; some specific questions focus on participants' familiarity with genetic terms; a series of vignettes examines issues of duty and obligations between family members. Forty young people and parents of young children have been interviewed to date.
The main research questions are:
a)What are the participants' concepts of inheritance and of the processes by which inherited characteristics are transmitted by parents to their children?
b)What are the participants' concepts of kinship and how are these related to concepts of inheritance?
This poster will illustrate the findings from interviews so far completed.

E-P44. Psychological well-being in individuals requesting pre-symptomatic testing for late-onset neurological diseases and controls

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Psychological issues are important for counselling and intervention in late-onset diseases, 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.
We studied 30 at risk-individuals (15 men/15 women), aged 18-70 (mean 26.9 years), who requested testing, when compared to 200 controls from the general population, using the "Psychological General Wellbeing" scale of Dupuy (22 itens, 6 subscales).
The study-group included persons at risk for familial amyloid polyneuropathy (24), Huntington disease (5) and Machado-Joseph disease (1). Both individuals at-risk and controls were currently healthy.
Pre-symptomatic individuals showed better indicators regarding (1) humour ($F=10,229$; $p<0.002$) and (2) health and wellbeing ($F=4,676$; $p<0.032$), but not (3) concern with health. This was in agreement with the item analysis, which was significant for self-control ($F= 10,991$; $p<0.001$), nervousness ($F=4,345$; $p<0.038$), melancholy ($F=4,759$; $p<0.030$), tension ($F=23,955$; $p<0.000$), sadness ($F=6,008$; $p<0.015$), restfulness ($F=5,148$; $p<0.024$), vigour ($F=20,058$; $p<0.000$), anxiety ($F=6,442$; $p<0.012$), happiness ($F=6,845$; $p<0.009$), and stress ($F=12,689$; $p<0.000$). In all these, the pre-symptomatic group had more favourable indicators of psychological well-being than controls.
One may have expected that individuals at-risk who came for pre-symptomatic testing were more concerned about their health and showed more adverse indicators regarding their psychological wellbeing. Our results, however, proved to be different, and may suggest (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.

E-P45. An appraisal of the German version of the Genetic Knowledge Index (GKI)

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Background: As more reliable and low-cost gene tests become available, knowledge of genetic concepts is increasingly relevant. Adequate understanding of gene technology is necessary to provide informed consent to genetic testing. Furthermore, genetic knowledge is necessary for participation in the public discourse about the further advancement of gene technology.
Purpose: In order assess lay people's basic genetic knowledge, Furr & Kelly (1997) developed the Genetic Knowledge Index (GKI). We translated the items of the GKI, and we aimed to investigate its usefulness in the German population.
Methods: The GKI which comprises five true-false-items was administered to N = 420 individuals. This complete sample consisted of three subsamples: n = 131 persons from the general population, n = 129 medical students, and n = 160 students from other faculties.
Results: The total score of the GKI discriminated between the three groups. However, the internal consistency of the GKI was very low. There were no significant correlations between the GKI score and external criteria (self-reported interest in genetics, self-evaluated general knowledge of genetics, attitudes towards genetic testing, and a genetic knowledge index based on questions from the medical state examination). Furthermore, the factorial solution was different from the original study.
Discussion: Because of the weaknesses in reliability and validity, the German version of the GKI seems not to be an appropriate tool for assessing genetic knowledge. Further refinement is necessary. We propose to add further items covering a wider range of genetics and thus to increase the content validity of the questionnaire.

E-P46. The behavioural phenotype of Bardet-Biedl syndrome

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Background:
Although behavioural characteristics, including disinhibited behaviour, an inability to recognise social cues and obsessive and compulsive tendencies, have been noted in individuals with Bardet-Biedl syndrome (BBS), to date no studies have systematically studied the behavioural phenotype.
Method:
Parents of 21 children with BBS seen for a multidisciplinary clinical assessment completed standardised measures of behaviour.
Results:
Children with BBS showed elevated levels of internalising problems including feeling withdrawn and anxious/depressed. They also had elevated levels of social, thought and attention problems. A significant minority scored in the clinical range on a measure of autistic symptoms, although none met clinical diagnostic criteria. The children also had elevated scores on a measure of repetitive behaviour and the majority was reported to be obsessive by their parents.
Conclusions:
These findings indicate considerable clinical need that had been unmet in many families. Further, they suggest that BBS has a characteristic behavioural phenotype that may be related to mutation type.

E-P47. Decision Making Factors Associated with Whether to Receive BRCA1/2 Genetic Test Results

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Study Design: Retrospective study of individuals offered research BRCA1/2 mutation analysis results.
Instrumentation: Self administered questionnaire assessing: 1) factors involved in the decision whether to receive results; 2) basic cancer genetics knowledge.
Participants in a breast/ovarian cancer family study were offered results from BRCA1/2 mutation analysis. 70 individuals (58%) completed this survey of which 46% chose not to receive their test results. The most important factor influencing the decision about receiving results was the desire for specific cancer risk information, followed by having information for children, concern about genetic discrimination for children, and use of results to help with cancer screening decisions. Using the information to assist in decisions about chemoprevention, prophylactic surgery, childbearing, concern about worry/anxiety, difficulty finding time to get results and competing demands of caring for an ill relative were ranked as less important. There were no differences in knowledge of basic cancer genetic concepts between those who received and did not receive results. Those who chose not to receive results considered concern that results might cause worry/anxiety and difficulty finding time for genetic counseling more important and obtaining specific cancer risk information less important (p values <.02). These results suggest that the desire to obtain more specific cancer risk information was the most significant factor associated with the decision to obtain test results. Concern about results causing worry/anxiety and finding time for genetic counseling were not ranked as important but were associated with the decision not to receive results. Implications for genetic counseling will be discussed.

E-P48. Changes in Approach to Children with Down syndrome in the Cyprus Society over the Past Seven Years.

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Cyprus is a small island in the eastern Mediterranean with a population of approximately 700,000. This constitutes it a small and rather closed society. In the past, differences were stigmatized and in particular individuals with Down syndrome tended to be isolated and institutionalized.
Seven years ago the general genetics services were established on the island and were offered primarily by a clinical geneticist and a genetic counsellor. Also through these new services a program of public education was developed. These two factors in conjunction were responsible for the change in perception of Down syndrome within society.
The majority of babies born with Trisomy 21 were referred to the genetic services so that support was offered initially and subsequently guidelines for early intervention were established. Most of these children are now attending regular schooling as opposed to being institutionalized. They are integrating well with their peers and the education system is slowly, but surely adapting to cater to their needs.
We believe that the same factors that caused the stigmatization, the size and closeness of the population, were beneficial to the rather quick change in approach of this society.

E-P49. Trisomy 16q: qualitative analysis of behaviour phenotype

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Trisomy 16q, a rare finding in clinical genetics, is yet poorly understood in terms of behaviour phenotype. Starting with a single case of partial trisomy 16q with dysmorphic, clinical, and cytogenetic traits, we undertook a detailed qualitative study of a behavioural profile of trisomy 16q. We performed the qualitative analysis of static images extracted from a video recording of a therapist-guided playwork was conducted in a structured environment according principles and philosophy of Maria Montessori. Our observation revealed that the tested persons were provided with a wide range of psychomotoric skills (the ability to follow movements of second persons or moving objects or to keep the attention focused despite involuntary movements or an ability to react to repeating invitations to act, sound or touch), social abilities (the ability to establish eye contact or the ability to display an outward reaction in response to demonstrated objects or the ability to approach other persons or objects) and emotional capacities (manifestations of proudness,

boredom or interests or the ability to focus the attention on sound or the ability to use the therapist's support). This knowledge on behavioural features and prospective development modalities of children with trisomy 16q is important for genetic counselling in order to determine the development prognosis.

E-P50. The Evolution of Clinical Genetic Referrals in Cyprus Over the Past Seven Years.

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Clinical genetic services, other than those offered for the Thalassemias, were established in Cyprus in 1995.
This clinic covers the whole population of the Cyprus Republic (population 700.00), and for a vast variety of disorders.
During the first years the referrals from physicians and other health professionals were limited. One of the primary reasons for this was probably a lack of relevant education. Gradually, there has been a significant increase in the rate of physicians requesting the services of clinical genetics. Referrals have increased particularly from the pediatric specialties and other health professionals caring for children with special needs.
We are presenting the data illustrating this increase and will discuss the factors that we believe have changed the approach of health professianals towards the clinical genetic services.

E-P51. Concept and Understanding of Disease in the Genetics Era

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The phrase 'genetic disease' is heard on a daily basis, but the concept 'genetic disease' is far from clear. Most human diseases have a complex aetiology, involving genetic, physiological, psychological, etc. factors on their causal pathway. Genetic explanations of a disease are highly context dependent (state of knowledge, background of nongenetic factors, study population). Even with a complete knowledge of aetiology a classification of diseases in terms of cause would not be satisfactory. Diseases have to be described in terms of aetiology as well as in the pathology and pathophysiology which results.
Six groups of students of medicine differing in their level of clinical and theoretical experience (1st, 2nd, 3rd and 6th year of medical education, 300 from the regular curriculum, 100 from the reformed curriculum) were interviewed by a structured questionnaire focussing on their concept of disease in general and genetic disease in particular, the latter understood as a causally oriented classification on the one hand and as an assignment to the specialty human genetics within the realm of medicine on the other.
Answers of study participants reflected the present prominence of human genetics for the diagnosis of monogenetic diseases and diseases with a well established mode of inheritance; they highly overestimated the importance of human genetics in the diagnosis of multifactorial diseases and in therapy and prevention of disease with a known genetic aetiology. The teaching of medicine has to place more emphasis on the development of a realistic estimation of human genetics for medical practice in general.

E-P52. Feeling at risk: How do women express themselves?

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Introduction
Although genetic risk counselling improves the accuracy of women's perceptions of the risk of developing breast cancer, many women still tend to over -or underestimate their risk. To understand the way in which women perceive their risk, we collected interview data of women who applied for genetic counselling. We assess how women verbalise their personal risk. Furthermore, we investigate how the risk information provided in the counselling influences the way women spontaneously express their risk. Finally, we inquire what being at risk for familial breast cancer means to these women.
Method
We collected data of 86 women who participated in a face-to-face interview after their genetic consult on breast cancer. Women were asked to describe their personal risk in their own words, and were invited to reflect on the meaning of the risk. In addition, the clinical geneticists completed a checklist to document the information provided in each individual consultation.
Results
Preliminary analysis shows that the majority of the women chose to use a verbal format to express their personal risk. Many women spontaneously mention negative emotions associated with the risk; "*I'm afraid to die young*"; or "*It's frustrating to go on living with an uncertainty like this*". Furthermore, many women reflect on actions to escape the risk: "*no evening passes by, without me checking [my breasts]*".
Discussion
We will discuss the results with respect to psychological distress and coping with the risk for familial breast cancer, and whether this implies recommendations for counselling.